M18358

Edgar March Crookshank

SAINT HILL, SUSSEX.

22101748953

PRESENTED BY
EDGAR M. CROOKSHANK, Esq.
SENIOR VICE-PRESIDENT
OF THE COLLEGE.
1925

THIS BOOK
IS THE PROPERTY OF THE
ROYAL VETERINARY COLLEGE
CAMDEN TOWN

With the Author's Compts.
13 April. 1891.

PULMONARY TUBERCULOSIS.

Digitized by the Internet Archive
in 2014

https://archive.org/details/b20401140

PULMONARY TUBERCULOSIS

ETIOLOGICAL AND THERAPEUTIC

BASED ON AN EXPERIMENTAL INVESTIGATION

BY

R. W. PHILIP, M.A., M.D., F.R.S.E.,

FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH; ASSISTANT PHYSICIAN TO THE ROYAL INFIRMARY; PHYSICIAN TO THE VICTORIA DISPENSARY FOR CONSUMPTION AND DISEASES OF THE CHEST, EDINBURGH.

EDINBURGH & LONDON:
YOUNG J. PENTLAND.
1891.

YOUNG J. PENTLAND : EDINBURGH, 11 TEVIOT PLACE ; LONDON, 38 WEST SMITHFIELD, E.C.

M18258

WELLCOME INSTITUTE LIBRARY
Coll. welMOmec
Call No. WF200
1891
P54p

PREFACE.

The following pages embody some of the results of a research begun in 1885 on the Etiology of Pulmonary Tuberculosis.

The first division was read before the Ninth International Medical Congress, Washington, 1887, and is reprinted from the Transactions. The experimental record, which follows, is published for the first time. The therapeutic portion was sketched primarily in illustration of the practical significance of the position adopted. It has been added to, as the result of further observation in upwards of a thousand cases, recorded during the three years succeeding the Washington meeting, up to last summer.

Since the earlier experiments were conducted the conclusions have been carefully reviewed, and, where it seemed desirable, corroborated by further experiment. Continued observation, both in the Laboratory and in Hospital and Dispensary practice, have emphasised the value of the etiological and therapeutic positions maintained; and I take this opportunity of thanking several physicians and pathologists, who have been good enough to communicate to me facts in illustration or support of those I have adopted.

It is hardly necessary to add that, since these papers were

placed in type, wider prospects in the therapeutics of tuberculosis have been revealed through the genius of him whose earlier successes in this field have been the means of stimulating all recent advances, and whose latest triumphs worthily overshadow all else in this department.

4 Melville Crescent,
Edinburgh, *March*, 1891.

CONTENTS.

PULMONARY TUBERCULOSIS.

I.—ETIOLOGICAL.

In the short time at my disposal,[1] I shall endeavour to give a brief résumé of a somewhat extended investigation with regard to the etiology of phthisis.

First of all, I must premise that the scope of the present paper is hardly indicated with sufficient strictness by the words "The Etiology of Phthisis." It is not my intention to discuss the morbid anatomy of the phthisical lesions, nor the dependence of the phthisical process on the presence of the tubercle bacillus, nor the important questions of heredity and of climatic and other influences, which figure so largely in the etiological chapter.

For the present, I start with an acceptance of the doctrine of the unity of the phthisical process and of the immediate dependence of the process on the presence of the bacillus. The rigidly exact observations and experiments of Koch and others have, in my judgment, placed this beyond doubt. I prefer, at least, not to raise the question now. But, in spite of the comparative fulness and clearness of our knowledge in these lines, it appears to me that we are far from a rational conception of the *actual cause of death* from phthisis. It was with the view of elucidating this further etiological problem that the present investigation was undertaken.

[1] Before the Ninth International Medical Congress, Washington 1887. See Transactions of the Congress, vol. i., "On the Etiology of Phthisis."

A glance through the literature of the subject reveals how seldom the attempt has been made to solve the problem, how comparatively seldom, indeed, the question has been raised. When the matter has been discussed, explanations have been offered which may be classified roughly under four heads, viz.: (1) Progressive asthenia. (2) Loss of hæmatosis. (3) The lighting up of fresh inflammatory foci. (4) The absorption of waste products.

Now, I have no desire to depreciate the value of these as integral factors in the process. My contention is that, in view of the comparative regularity of the clinical phenomena, and in the light of more recent work, they do not afford sufficient explanation. Each of them was fully discussed prior to the discovery of the tubercle bacillus; and Jaccoud, more especially, has the credit of emphasizing the importance of the fourth, namely, the absorption of waste products. Since the announcement of the tubercle bacillus, comparatively little has been added in this direction, though the features and clinical course of an ordinary case of phthisis, and those of experimentally induced tuberculosis, are well defined and strikingly similar.

What, then, is the *modus operandi* of the tubercle bacillus in leading toward death?

Its fatal propensities cannot, I think, be regarded as merely irritant or privative. In all probability, they are attributable to a power possessed by it of elaborating new products, which are afterward absorbed.

Before explaining on what facts I base that statement, I ought to mention that Dr. Hermann Weber has hinted at the probability of such elaboration and absorption.[1] In the Croonian Lectures (1885), Dr. Weber speaks of "the chemical poison which probably is originated by the development of the tubercle bacillus in the tissues in an analogous manner, as, according to Gaspard, Panum, Billroth, Burdon Sanderson and others, a powerful chemical poison—sepsin—is developed in the process

[1] Mr. Watson Cheyne also (1885) expresses his belief "that this view of the production of a poisonous substance by the bacilli may explain the fever and wasting of phthisis."

of septicæmia." I am not aware, however, that up to the present time any attempt has been made to treat the matter more seriously. Whether this supposititious product or products be secreted by the bacillus, or be elaborated from the tissues which it infests, raises another question, which must be discussed later. It is enough, meanwhile, if we recognise the probable dependence of these new products on the presence and action of the bacillus.

Such a process of elaboration or secretion has its analogue in the more evident varieties of fermentation which have been studied by Pasteur, Schützenberger, and others, for example, the alcoholic, the lactic acid, the butyric acid, and the ammoniacal. More particularly the view appears to me substantiated by the following weighty evidence :—The association of special forms of microzymes with special forms of fermentative action has been conclusively demonstrated by Pasteur and a large school of subsequent observers. A distinct variety of fermentation as certainly follows the admission into a suitable medium of a given microbe as the exclusion of the same microbe excludes the possibility of its occurrence. Further, the rearing of pure cultivations has shown that different effects are obtained, and, certainly, marked differences in the rate of growth are observed, according to the constitution of the medium in which the cultivation is attempted, while certain organisms are most exclusive in their selective affinities. Moreover, if the same medium, say Koch's gelatine, be utilised for the cultivation, in different tubes, of different micro-parasites, the effects produced on the medium are very different in the several instances. Even in the gross, differences, for example, in the rate and amount of liquefaction and in the production of certain gases, are marked. And it is in the highest degree probable that careful examination of the medium, after cultivation has been carried out for some time, would show important alterations in its chemical constitution, such as occurs in the better known forms of fermentation.[1] In other words, the

[1] Since this was written, in January 1887, much important work has been done along these lines, to which reference need not be made at present.

living organism has the power of disturbing, or rather, in order to the preservation of its own life, the organism is compelled to disturb, the molecular arrangement of the elements in the medium of cultivation.

These considerations open up a wide and promising field for investigation. This appears to me the aspect of bacteriological observation which is pregnant with most important results. In illustration of this, the work of Panum, Selmi, Gautier, Bergmann, Schmiedeberg, Brieger, and others, need only be cited.

In practically applying this hypothesis to the problem of phthisis, I directed my attention first of all to urine. The results obtained, which have been given elsewhere, were not sufficiently definite in character to warrant their citation here. Examination of portions of the diseased organs, or of their glandular appendages, was abandoned, as it was found difficult to have these sufficiently fresh to avoid the objections that would inevitably assail successful results so attained. This led to the adoption of the sputum as the *materies morbi* for investigation, and that on the following, among other, grounds:—

(1) The sputum is the constant accompaniment of the morbid condition, and stands in a peculiar relationship to the diseased organs.

(2) It is accessible in large quantity, fresh, and therefore, as much as possible, free from such contamination as might be supposed to introduce fallacy.

(3) It has been shown that the maximum amount of the contagious element resides in the sputum.

(4) Having regard to the conditions of growth of the tubercle bacillus, it seems likely that the muco-purulent secretion is a peculiarly good medium for cultivation.

(5) It has been proved that tubercular sputum retains its virulence for months.

(6) The presence of the tubercle bacillus can be comparatively easily determined, while, with greater care, its relative abundance in different specimens may be gauged.

(7) The sputum can readily be subjected artificially to similar conditions outside the body as within the chest.

(8) Much of the experimental work already carried out with reference to tuberculosis has been done by the subcutaneous and intra-venous injection of unaltered phthisical sputum. (Villemin, Chauveau, Biffli, Veza, Semmer, Tappeiner, etc.)

(9) Collateral evidence, from the side of some ptomaine investigations, seems to imply that the ready access of oxygen to the centre of ptomainic production may aid in their development.

After approaching the subject in a variety of ways, with a remarkable constancy of results, I thought it best to institute a series of experiments, with extracts obtained from different phthisical sputa, by such methods as could be least open to objection in respect of complications introduced from without.

The sputum was carefully collected in a clean vessel, preferably a closed jar with central hole for the entrance of the expectorated material, such as is used in some of the Edinburgh Royal Infirmary Wards. In the selection of the patient the greatest care was exercised. (*a*) Only such cases were made use of as showed undoubted signs of advancing phthisis. (*b*) No case was accepted where the temperature chart did not record a more or less persistent elevation. (*c*) After the first two or three examinations it was found best to restrict the selection to subjects where possible impurities from smoking were absent.

Similar care was taken in the selection of the sputum. (*a*) The sputum was rejected when any foreign admixture was present, such as vomited materials. (*b*) It was rejected when saliva was present in appreciable amount. (*c*) The reaction of the sputum was tested, and only such admitted as gave an acid or neutral reaction. This last condition was found always associated with a peculiar odour, which may be regarded as *sui generis*. (*d*) The presence, and approximately the relative

abundance, of the tubercle bacillus was in every instance ascertained.

Method.—The sputum thus carefully collected for 12 or 24 hours, is at once subjected to further examination. Its bulk is measured, and three volumes of rectified spirit are added to it. The mixing process is carried out *guttatim*, so that the separation of the elements of the sputum may be rendered complete, and the admixture made as intimate as possible. If the sputum be neutral or but faintly acid, a trace of tartaric acid is added to the rectified spirit previous to mixing. The whole is transferred to a Florence flask. Its mouth having been protected by a fine muslin rag, the flask is placed in a Koch's steam sterilizer and exposed to a gentle, moist temperature of 36°-40° C. for 20-24 hours. At the end of this time the fluid is carefully filtered, first, once or twice through fine muslin, and then three or four times through filter paper, till the filtrate runs off perfectly clear. Its volume is then measured, and the whole evaporated down in open beakers to $\frac{1}{15}$ of its bulk (*circa*). This reduces it to the consistence of a more or less muddy extract, varying in colour according to that of the original sputum. The latter part of the process is conducted slowly, with the view of driving off all remaining trace of spirit, and to prevent the escape of more volatile products. The extract thus obtained was utilised for injection.

With regard to its constitution, it must be observed that it is as pure an extract as can well be obtained of the carefully selected sputum. The only additions made are measured quantities of faintly acidulated rectified spirit. This, in the process of slow evaporation to $\frac{1}{15}$ its original volume, was presumably entirely given off, so that in observing the results we have to deal with the effects of a fairly purified extract of phthisical sputum, *i.e.*, sputum minus the coagulable elements, separated out by the addition of the rectified spirit and the after process of filtration. It should be mentioned further, that the extract, when properly prepared, is most unstable, and being extremely liable to the attack of fungi, breaks down in the course of a few days, giving rise to new products. The extract, therefore, was

never used for experimental purposes after it had been prepared for three or four days.

Four series of experiments were conducted with the extract so obtained.

(1) To observe its effects on the system generally.

(2) To observe its effects on the circulation, *i.e.*, on the cardiac rate.

(3) To test the antagonistic effects of certain drugs, especially atropine, as regards the system generally.

(4) To test these antagonistic effects as seen more especially in the cardiac rate.

It is impossible here to give details of the numerous experiments conducted under these heads,[1] but the general results may be summarised.

Series 1. Effects on the System Generally (Exps. I.—XXII.).

(*a*) *On Frogs.*—Thirteen experiments, carried out with varying quantities and under a variety of conditions, yield results of striking uniformity, and point to the presence in the extract of a toxic principle, or of toxic principles, of considerable potency. The results differ only in degree, a progressive increase in the intensity of the symptoms being observable with the increased dosage. The general line of symptoms is that of a gradual development of voluntary motor depression. In no instance was a stage of excitation traceable. This condition of depression appeared, in part, explicable by a toxic influence exerted on the higher nerve centres. This is evidenced by the general character of the depression, by the sluggish nature of the movements, while co-ordination was little disturbed, and by contraction of the pupil (?). The spinal cord appeared to be little affected, the reflexes remaining normal throughout in the less severe cases, and in the graver, being unaffected till later on.

(*b*) *On Mammalia* (Exps. XIV.—XXII.)—In mice it was found possible to induce distinct symptoms with ·3 cc. of the extract. They resembled in general character those in the frog, and

[1] A more extended record of the Experiments follows, pp. 13–34.

passed off gradually in the course of an hour or two. With increased injection, the intensity and duration of the symptoms were correspondingly increased. As in the frog, the scope of the symptoms suggests implication more especially of the higher centres. There was the same early appearance of gradually advancing depression. This, as before, was not preceded by any trace of excitation. In the course of ten minutes, the animal invariably became quieter, the stage of quiescence passing on to more or less complete passivity and disinclination for movement, according to the amount injected. In the lighter cases, this was recovered from. In the more severe cases, it deepened into death; or death followed after more or less complete approach toward recovery. In addition to these symptoms, common to frogs and mice, certain well-marked phenomena were observed. Among the more striking of these should be noted fibrillary twitching of the surface of the body and convulsive movements of the trunk and limbs. Regarding changes in the respiration, it has to be borne in mind that the estimation of the rate of breathing is always difficult in mice. The general impression, however, was that after the preliminary excitement there remained a certain increase in the respiratory rate, to be followed later, when symptoms were sufficiently prolonged, by retardation. In those animals which died after prolonged symptoms, anorexia was a conspicuous feature, while water was drunk freely.

In rabbits comparatively large quantities of the extract were required to produce urgent symptoms. On economic grounds this line of experimentation was less systematically carried out. So far as they go, the results obtained were in strict accord with those just detailed. Of greater interest, however, in the case of the rabbit, was the effect of daily repeated small doses. Thus, for example, two rabbits were fed on measured quantities of oats and water, and their weights registered for some days until the daily register became fairly constant. The same conditions were continued, with the addition that once in the twenty-four hours each animal received subcutaneously small injections of the extract. Presumably as a result of this, their

weight progressively decreased by amounts varying from ¼ oz. up to 1 oz. *per diem*, and the amount of food consumed was reduced to one-half, and on one occasion to one-quarter the amount previously consumed in the corresponding time. After some days the system appeared to grow more tolerant of the morbid material, as it was found necessary to increase the dose to produce the same effect. At the end of ten days the injections were discontinued, and the weights, without increasing, remained almost constant for a week or two. Then a gradual progression downward, apart from fresh injection, was observed, each animal continuing to lose a fraction of an ounce daily until death. It appears likely that the early loss of weight was due directly to the action of the morbid product, which doubtless led to loss of appetite, &c. This is evidenced by the daily loss of weight, corresponding with the dates of injection, and by the return to a more constant condition when the injections were stopped. The later progressive loss of weight, apart from injection, is more difficult of explanation. We may suppose that, following the earlier injections, a condition of marasmus developed. In neither of the rabbits was there found, on *post-mortem* examination, the slightest trace of caseation, to which rabbits are prone.

Series 2. Effects on the circulation, *i.e.*, on the cardiac rate (Exps. XXIII.—XXVIII.).

A considerable number of experiments were conducted under this head. They prove conclusively the presence of a powerful cardiac depressant. In each instance the fall is striking. Where large doses were used it is remarkable; the cardiac rate being reduced under the influence of the extract from 44 per minute to 18 and even 14 in the course of four hours. Coincident with the decrease in rate, a marked lengthening of the diastolic in relation to the systolic phase was evident. These results, taken along with those of Series 4 *(infra)*, imply, I think, that the depressant action on the heart is produced through the medium of the inhibitory fibres, and not by direct action on the cardiac ganglia.

SERIES 3 AND 4. EXPERIMENTS TO SHOW HOW (*a*) THE GENERAL, AND (*b*) THE SPECIAL ACTION OF THE EXTRACT IS OPPOSED BY ATROPINE (EXPS. XXX.—XLIII.).

It is convenient, in this brief summary, to combine the results obtained in Series 3 and 4. In each, it was endeavoured to neutralize the ascertained depressant effects of the extract by the exhibition of presumably antagonistic drugs. For the present, I limit myself to the results obtained with atropine. The double series yield results in remarkable consonance with those obtained in the earlier series. In the first place, they afford strong corroborative evidence as to both the general systemic and the special cardiac effects of the extract. But, in the second place, they prove that the combined exhibition of atropine undoubtedly modifies these results in a striking manner. Of this there is evidence in all the experiments, the degree to which such modification is produced varying with the relative quantity of the antagonistic principle. Most perfect antagonism was produced by the combined injection of $\frac{1}{60}$ milligramme sulphate of atropine with ·6 cc. extract (Exp. XXXVIII.) Under such conditions, the general systemic effects, easily produced both in frogs and in mice by ·6 cc. extract, were almost completely absent, while the cardiac rate, which ·6 cc. sufficed to depress considerably, remained practically constant. The effects were similar whether the atropine was exhibited simultaneously with the extracts or at varying intervals before or after. The antagonising influence of atropine is most strikingly demonstrated in those experiments where the injection of the extract preceded that of the atropine by a measured interval of time. In such cases the effects of the extract were, first of all, well defined, and gradually declined on the addition of the atropine. Similar results, though less striking, were obtained when the atropine preceded the extract. It should be added, that in every instance where counter-experiments were made with atropine, the extract was first tested, with the view of establishing its physiological action.

This experimental record is necessarily too brief, and doubtless

is open to much criticism. But the results at my disposal, which I hope to publish in more extended form, appear to me to justify the statement that from the tubercular sputum there is separable one or more products possessed of well-marked toxic properties, these toxic properties being more or less completely opposed by atropine.

The remaining question is, In how far this poisonous principle is dependent on the presence of the bacillus? Might not such toxic effect be produced by extracts obtained from other sputa besides those strictly bacillar? There is, unfortunately, no time to give in full the grounds for my statement, and I must content myself with stating categorically my belief, formed on experimental grounds, that the presence of the bacilli is causally related to the poisonous product obtained from the sputum. I incline, also, for similar reasons, to the belief that there is a relation traceable between the toxicity of the extract and the abundance of the bacillar elements discoverable in the sputum.

On the question of absorption and the therapeutic indications, regarding which I had proposed speaking, I must not dwell.

But it may be convenient, in closing, to tabulate shortly the chief points which have been considered.

(1) In view of the work of Koch, it is impossible to avoid admitting that a causal relationship exists between the tubercle bacillus and the phthisical process.

(2) The mere predication of this relationship is not sufficient in explanation of the clinical facts and the generally fatal termination of such cases.

(3) The usually received explanations of the *modus moriendi* in phthisis are insufficient.

(4) It appears probable that the lethal influence of the bacillus is due to the production thereby of certain poisonous products.

(5) Clinical and experimental evidence appear to indicate that the morbid secretions from the respiratory surfaces afford a good medium for the growth of the tubercle bacillus, and, presumably, for the elaboration of such products.

(6) Such a product is separable from the carefully selected and prepared sputum.

(7) This product is possessed of well-marked physiological properties, being eminently toxic to frogs, mice, and other animals.

(8) The toxic properties of the product are, speaking generally, depressant.

(9) More particularly, they include a markedly depressant influence on the heart.

(10) This depressant influence seems to be exerted through the medium of the cardio-inhibitory mechanism.

(11) The toxic action of the product is more or less completely opposed by atropine.

(12) The amount of the product which may be separated appears to bear a distinct relation to the abundance of the bacillar elements present.

(13) Absorption of the poisonous product most probably occurs by way of the lymphatic circulation.

EXPERIMENTAL RECORD.

The following condensed record of experiments is arranged in the order referred to in the preceding statement :—

Series 1. Effects on the system generally.

Series 2. Effects on the circulation, *i.e.*, on the cardiac rate.

Series 3. Antagonistic effects of Atropine, as regards the system generally.

Series 4. Antagonistic effect of Atropine, in respect of the circulation.

Series 1. Experiments to show the action of the extract on the system generally—A. On Frogs.

B. On Mice.

SERIES 1.—(A.) ON FROGS.

Exp. I.—Injection of ·25 cc. prepared extract, diluted with ·37 cc. distilled water, into posterior lymph sac of medium size, lively frog (R. temp.) Pupils medium.

1 p.m.	*Injection of ·25 cc. diluted as above,*
1.5	In statu quo.
1.10	There has been no change.
1.15	Less lively. Jumps about less. Seems to prefer remaining in one place.
1.20	Remains quiet. When irritated, jumps sluggishly, and on landing, head tends to fall forwards.
1.25	Sits perfectly still in corner. When irritated, crawls, rather than jumps away.
1.30	Lies almost flat, with limbs relaxed. Responds but slightly to mechanical stimulus. If turned on back, makes feeble resistance and only regains ordinary position after a minute or two. No marked alteration of pupil has been observed.
1.45	For last quarter of hour, has remained in similar condition.
2.	In statu quo.
2.30	In statu quo.
2.45	In statu quo.
3.	Looks a little brighter.
3.30	Has recovered slightly. Makes effort at progression forwards.
4.	Gradual signs of return to ordinary condition.
6.	In course of last hour, gradually returned to normal state.

Exp. II.—Injection of ·4 cc. prepared extract, diluted with ·4 cc. distilled water, into posterior lymph sac of rather larger lively frog (R. temp.) Pupils medium.

12.55 p.m.	*Injection of ·4 cc. diluted as above.*
1.	There has been no change.
1.5 p.m.	Has jumped into corner, where lies comparatively quietly. Looks less lively. Reacts freely to mechanical stimulation, but less energetically. Jumps for short distance, if irritated. Movements seem well co-ordinated.
1.10	When irritated, jumps less readily. Tends to crawl. Becomes still, when stimulus ceases. Head tends to droop. Body more or less completely prone.
1.15	Can scarcely be made to jump with strong mechanical stimulus. If turned on back, makes but feeble and unsuccessful effort to return to ordinary position. Pupil is slightly contracted.
1.20	Remains on back. Pupil more evidently contracted.
1.25	In statu quo.
1.28	After effort, rights itself.
1.30	Lies on back. Reflexes normal.
1.40	In statu quo.
1.50	Makes effort at recovering position. When stimulated mechanically, makes no effort at movement.
2.	In statu quo.
2.10	Lies on back. Effort at recovering position more frequent.
2.20	In statu quo.
2.30	Signs of slight improvement. Makes occasional effort to crawl forward, but rapidly returns to lethargic condition.
2.45	Signs of improvement more pronounced.
3.	Improvement continues.

Observations were continued throughout the afternoon, and showed gradual return in course of succeeding hour to normal state. Next day as lively as before injection.

Exp. III.—Injection of ·6 cc. prepared extract, diluted with ·6 cc. distilled water, into posterior lymph sac of large, lively frog (R. temp.) Pupils medium.

Exp. III.—*continued*

1 p.m.	*Injection of ·6 cc. diluted as above.*
1.5	No change detected.
1.10	Little change. Jumps freely when stimulated.
1.15	Less lively. Disinclined for movement.
1.20	Jumps sluggishly into corner and lies still. Mechanical stimulus has little effect. Movements seem well co-ordinated.
1.25	Crawls, rather than jumps, when irritated. Lies in corner, prone. Head droops. Reflexes appear normal.
1.30	Lies still. If turned on back, lies continuously so. Makes but slight effort to recover position. Pupil slightly contracted.
1.40	In statu quo. Lies indifferently on back or prone.
1.50	In statu quo. Pupils distinctly contracted.
2.	In statu quo.
2.30	In statu quo.
3.30	For last hour in statu quo. From time to time signs of improvement appear.
4.	In statu quo. Pupils less contracted.
5.	In statu quo.
6.	Still continues to lie on back, without offering much resistance.

Throughout the evening, gradual improvement occurred. Next day, appears as lively as ever.

Exp. IV.—Injection of ·6 cc. prepared extract, diluted with ·4 cc. distilled water, into posterior lymph sac of medium sized frog (R. temp.) Pupils medium.

1 p.m.	*Injection of ·6 cc. diluted as above.*
1.5	In statu quo.
1.10	Appears quieter. Jumps readily if irritated.

Exp. IV.—*continued.*

1.15 p.m.	Markedly quieter. Prefers to remain still in corner, with tendency towards prone position.
1.20	Lies almost flat, with head drooping and only occasionally elevated. When stimulated crawls slowly off and speedily comes to rest. Pupils less, reflexes appear normal.
1.30	There has been no change, but symptoms of depression more marked.
1.35	Lies indifferently in any position.
1.45	If turned on back, continues in position without offering resistance. Pupils more contracted.
2.	In statu quo.
2.30	In statu quo. Lies perfectly still on back.
3.	In statu quo.
4.	In statu quo.
4.30	Gradual signs of improvement. Makes effort at recovering position. Pupils less contracted.
5.	Symptoms appear to be passing off.
5.30	Continues to improve. Makes effort to jump from time to time.
6.	Has largely recovered normal condition. Movements continue less active.

Next morning, appears as lively as ever.

Exp. V.—Injection of ·6 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils moderately dilated.

12.25 p.m.	*Injection of ·6 cc. undiluted.*
12.30	In statu quo.
12.35	Appears quieter. Occasionally makes short jumps. Tends to remain still in corner.
12.40	Scarcely re-acts to moderate irritation. Crawls sluggishly.
12.45	When irritated, moves off sluggishly to corner. Reflexes appear normal.

Exp. V.—*continued.*

12.55 p.m.	In statu quo. Still quieter.
1.	If turned on back, offers very feeble resistance. Pupils less.
1.5	Lies continuously on back. When placed on face lies quite flat.
1.10	In statu quo.
1.20	In statu quo.
1.40	In statu quo.
2.	In statu quo. Pupils more contracted.
2.15	Very marked depression. Reflexes appear normal.
2.30	In statu quo.
3.	In statu quo.
3.30	In statu quo.
4.30	In statu quo. Still lies indifferently on back or face.
5.10	In statu quo.
5.20	Slight signs of improvement appearing. Makes faint effort at recovering position. Pupils less contracted.

The experiment was here interrupted, that certain other effects might be observed.

Exp. VI.—Injection of ·9 cc. prepared extract undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium.

1 p.m.	*Injection of ·9 cc. undiluted.*
1.5	In statu quo.
1.10	Evidently less lively. Only jumps when stimulated. Prefers to lie in corner.
1.15	Lies perfectly still, almost flat, with head drooping. When irritated makes slow crawling movement forward. Pupils less.
1.20	Depression deepens.
1.25	Very marked depression.
1.30	Lies indifferently in any position. If turned on back, makes no effort at recovery. When stimulated makes no attempt to turn. Reflexes appear normal.
1.40	In statu quo.
1.50	In statu quo. Pupils more contracted.

Exp. VI.—*continued.*

2 p.m.	In statu quo.
2.30	In statu quo.
3.	In statu quo.
4.	In statu quo.
5.	In statu quo.
5.30	Depression appears rather less. When turned on back makes feeble effort at recovering position.
6.	Appears rather brighter. Still much depressed. Pays little attention to irritation.
7.	In statu quo, but offers more resistance when turned on back.
7.30	Gradual signs of improvement.
8.	Improvement continues.

On the following morning, still remains rather quieter than normal. Otherwise seems to have recovered.

Exp. VII.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium.

5.35 p.m.	*Injection of ·9 cc. undiluted.*
5.40	There has been no change.
5.45	In statu quo. Seems quieter.
5.50	Less lively. Appears disinclined for movement.
5.55	Distinctly quieter. Lies flat, with head drooping. Movements are slight, sluggish, and of crawling character. Pupils less. Reflexes seem normal.
6.5	Lies indifferently in any position. When turned on back, offers no resistance. Pupils more contracted. Reflexes appear less active.
6.15	Depression very marked.
6.25	As before, lies passive in any position.
6.35	In statu quo.
7.	In statu quo.
8.	In statu quo.
9.	In statu quo.
10.	In statu quo. Left lying on back.

Exp. VII.—*continued.*

On following morning (9 a.m.), does not appear much brighter. Pupils remain less than before. The symptoms slowly disappear during the day. On the third morning, frog appears well.

Exp. VIII.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium.

5.30 p.m.	*Injection of ·9 cc. undiluted.*
5.35	No change.
5.40	Appears depressed, jumps less freely.
5.45	Lies in corner, with head drooping.
5.50	Lies almost flat. Makes little attempt at change of position, even when stimulated. When movement occurs, it is dragging or crawling in character. Pupils slightly contracted.
5.55	Lies indifferently in any position. Makes no effort at resistance when turned on back. Reflexes appear normal.
6.	In statu quo. Pupils distinctly less.
6.30	In statu quo.
7.	In statu quo.
8.	In statu quo.
9.	In statu quo.
10.	In statu quo. Left lying on back.

On following morning (9 a.m.), in much same state. Lies indifferently on back or abdomen or side. Pupils still somewhat contracted. Throughout the day the condition gradually improves, but on the morning of the third day, the frog remains slightly affected and disinclined for movement.

Exp. IX.—Injection of 1·2 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium.

6 p.m.	*Injection of 1·2 cc. undiluted.*
6.5	Little appreciable change.

Exp. IX.—*continued.*

6.10 p.m.	Severe depression. Lies perfectly flat with head drooping. Even when irritated strongly, jumps away slowly, and in incomplete fashion. Pupils slightly contracting.
6.15	Depression still deeper. Lies passive.
6.20	Perfectly helpless. Lies indifferently on back or side without making effort to recover position.
6.30	In statu quo. Pupils less.
6.45	In statu quo. Reflexes appear diminished.
7.	In statu quo. Gravest state of depression yet witnessed. Lies as if dead, except for respiratory movements.
7.30	In statu quo.
8.	In statu quo.
9.	In statu quo.
10.	In statu quo. Depression continues very great.

On following morning, found dead.

P. M. Examination.—Pupils contracted. No rigor mortis. Heart in diastole. Other organs appear normal. No sign of irritation at seat of injection.

N.B.—*In the succeeding four experiments, the effect of repeated doses was observed.*

Exp. X.—Injection of ·6 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium. *Followed*, after interval of five hours (*circa*), by similar injection.

12.25 p.m.	*Injection of ·6 cc. undiluted.*
12.35	Signs of commencing depression.
12.45	Movements sluggish and crawling in character.
12.55	Lies still in corner. When irritated, makes sluggish movement for short distance. Pupils less. Reflexes normal.
1.10	Lies indifferently in any position.
1.30	In statu quo.
2.30	In statu quo.
3.30	In statu quo.

Exp. X.—*continued.*

5 p.m.	Slight trace of improvement, but is still much depressed.
5.15	*Injection of ·6 cc. undiluted.*
5.20	In statu quo. Occasional crawling movement forwards.
5.30	Depression deepening, lies quite passive.
5.45	Very grave depression.
6.	In statu quo.
7.	In statu quo.
8.	In statu quo.
10.	In statu quo.

On the following morning it was found in similar state, lying passive as if dead, till

5. p.m.	When neither respiration nor cardiac beat could be detected.
5.10	In statu quo. Dead.

P. M. Examination.—Pupils moderately contracted. Rigor mortis absent. Heart in diastole. Other organs seem normal. No sign of irritation at the seat of injection.

Exp. XI.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium. *Followed*, after interval of five hours (*circa*) by similar injection of ·6 cc. undiluted.

12.20 p.m.	*Injection of ·9 cc. undiluted.*
12.25	No change.
12.30	Distinctly quieter. Lies in corner.
12.35	Appears disinclined to move. When stimulated, moves sluggishly away in crawling manner.
12.40	Lies flat on face. Head drooping. Pupils less.
12.45	When stimulated, occasionally makes forward crawling movements.
12.50	In statu quo. Reflexes seem normal. No increased diminution of pupils.
1.	Grave depression.
1.10	In statu quo.
1.20	Lies indifferently on back or abdomen without resistance. Pupils distinctly contracted.

Exp. XI.—*continued.*

1.30 p.m.	After lying on back for long period, occasionally makes slow effort at recovery of position.
1.45	Depression deeper. Lying on back passive. Pupils still more contracted. Reflexes appear normal.
2.	In statu quo.
2.30	In statu quo.
3.	In statu quo.
4.	In statu quo.
5.	Suggestion that depression is passing off slightly. When irritated reacts slowly and clumsily.
5.10	*Injection of ·6 cc. undiluted.*
5.20	Depression more marked.
5.30	Lies indifferently in any position, pupils contracted.
6.	In statu quo.
8.	Has continued in same condition.
10.	During last two hours no change of importance observed. Left lying passive on back.

On the following morning in much the same condition. It continues in similar state for twelve hours, depression gradually becoming more marked till death about 9 p.m.

P. M. Examination.—Pupils slightly contracted. No rigor mortis. Heart in diastole. Other organs normal. No irritation at the seat of injection.

Exp. XII.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog. (R. temp.) Pupils medium. *Followed*, after interval of five hours (*circa*), by similar injection of ·9 cc. prepared extract undiluted.

1.10 p.m.	*Injection of ·9 cc. undiluted.*
1.20	Apparently some hesitancy in jumping.
1.25	Less lively. Not affected by ordinary mechanical irritation. Reflexes appear normal, pupils less.
1.30	Lies flat. When irritated, makes no effort at jumping.

Exp. XII.—*continued.*	
1.35 p.m.	Almost passive. Lies indifferently on back or face.
1.45	In statu quo.
2.	Depression marked.
2.30	In statu quo.
3.30	When left for some time on back, makes occasional feeble efforts to recover position.
4.30	Severe depression continues.
5.30	In statu quo.
6.	Looks rather brighter, but depression still marked. When irritated, makes occasionally crawling effort forwards.
6.5	*Injection of ·9 cc. undiluted.*
6.15	Depression deeper. Pupil distinctly contracted.
6.20	Lies as if dead, except for respiration.
6.30	In statu quo.
7.	In statu quo.
8.	In statu quo.

On following morning (9 a.m.), found dead.

P. M. EXAMINATION.—Pupils contracted. No rigor mortis. Heart inadvertently injured in opening chest. Other organs normal. No irritation at seat of injection.

In the following experiment the repeated injections were made at greater intervals.

Exp. XIII.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog (R. temp.) Pupils moderately dilated. *Followed* in twenty-four hours by a similar injection of ·9 cc., and in twenty-two hours more by injection of ·6 cc. undiluted. (The report is condensed.)

First day.	
1.30 p.m.	*Injection of ·9 cc. prepared extract, undiluted.*
1.40	Signs of commencing depression.
1.50	Depression marked. Prefers to lie in corner. Movements are infrequent and of crawling, rather than springing character. Pupils not markedly altered.

Exp. XIII.—*continued.*	
2. p.m.	Lies almost perfectly flat, with head drooping. Remains passive to ordinary mechanical irritation.
2.15	Absolutely passive. No attempt at voluntary movement. Lies indifferently in any position. Pupil varies at times, being contracted, and then less evidently so.
2.30	In statu quo.
3.	Depression grave.
4.	Appears rather brighter, but depression remains serious.
6.	In statu quo.
8.	In statu quo.

On the following morning, found lying flat on abdomen as left. When irritated, re-acts more easily. Pupils medium. This state continued throughout forenoon with gradual improvement.

Second day.	
1.30 p.m.	*Injection of ·9 cc. undiluted.*
1.40	Increase of depression.
1.50	Depression again very marked. Lies indifferently in any position. Pupils less.
2.	In statu quo.
2.30	Lies as if dead. Respiration and cardiac beat hardly detectable. Mouth hangs open.
3.30	In statu quo.
4.30	Slight improvement evident, makes occasional movement, and after much effort turns from back on to abdomen.
5.30	Has continued to lie on back for past half hour.
6.30	In statu quo.
8.	In statu quo, rather brighter.

On the third morning, appears brighter, though still evidently depressed. Movements continue sluggish, but objects to lie on back. This improvement became more marked throughout forenoon. Pupils moderately dilated (11.30 a.m.).

11.40 a.m.	*Injection of ·6 cc. undiluted.*
11.50	Evident deepening of depression.
12.	Once more lies indifferently on back or abdomen. Pupils less.

Exp. XII.—*continued.*

12.15 p.m.	Lies as if dead. Pupils more evidently contracted.
12.45	In statu quo. Very severe depression.
2.	Gradually shows signs of improvement.

This experiment was continued with smaller doses, the effects of each injection being correspondingly less. When the injections were discontinued the frog recovered perfectly.

Series 1.—(B.) On Mice.

Exp. XIV.—Injection of ·3 cc. prepared extract into medium-sized, lively mouse.*

10.50 a.m.	*Injection of ·3 cc.*
10.55	In statu quo.
11.	Has become quieter, looks frightened. No longer runs about. Prefers to remain still in corner. When touched, runs for short distance. Movements perfectly co-ordinated.
11.5	Looks depressed and anxious. Head no longer erect.
11.15	In statu quo. There has been no change.
11.20	Continues to lie in corner, with head resting on table. When irritated, moves forwards for short distance in regular fashion, but soon relapses to condition described.
11.30	In statu quo.
11.35	Appears rather less depressed. Occasionally raises head and nibbles at oats.
11.40	In statu quo.
11.45	Improvement continues.
11.50	Appears recovering gradually.

* Experiments were made on house mice throughout. The injections were made subcutaneously.

Exp. XV.—Subcutaneous injection of ·6 cc. prepared extract into medium-sized, lively mouse.

Exp. XV.—*continued.*

11.5 a.m.	*Injection of ·6 cc.*
11.10	In statu quo.
11.15	Has run to corner, where remains perfectly quiet.
11.20	Looks depressed and frightened. Lies almost prone, with head depressed.
11.25	Lies in same position. Respiration, always rapid, appears increased.*
11.30	In statu quo. Occasionally fibrillary twitching occurs over surface of body.
11.35	Twitching continues from time to time. During last five minutes, has made one or two spontaneous movements forwards, perfectly co-ordinated, speedily settles down to comparatively passive state.
11.45	In statu quo. There has been no change.
11.50	No fibrillary twitching for last ten minutes. Depression appears less deep. Still lies with head resting on table.
12.	Depression appears less.
12.15 p.m.	During last quarter of hour, signs of gradual improvement.
12.30	Improvement continues. Seems gradually recovering.

* Not much stress is placed on this, as the respiration in mice varies much and is not easily counted.

Exp. XVI.—Subcutaneous injection of ·9 cc. prepared extract into medium-sized, lively mouse.

12.15 p.m.	*Injection of ·9 cc.*
12.20	In statu quo.
12.25	Has crouched into corner. Looks frightened.
12.30	Lies in corner, with head depressed on table. No attempt at spontaneous movement. Respiration appears quickened.
12.35	In statu quo. Occasionally makes short movement forward. Movement is regular and co-ordinated. Speedily comes to rest again.

Exp. XVI.—*continued.*

12.40 p.m.	Fibrillary twitching, slight, occurs from time to time. General convulsive movement of abdomen occasionally, resembling effort of vomiting.
12.45	In statu quo. When irritated, makes short, slow movement forward. Movement is fairly co-ordinated.
12.55	In statu quo. Is almost completely passive even when irritated. Is with difficulty made to shift position, and when irritation removed, settles down into old state. Respiration quicker.
1.5	In statu quo.
1.10	Trembling of whole body from time to time.
1.20	Breathing less rapidly, looks rather brighter.
1.30	Signs of slight improvement. Occasionally raises head to nibble at oats.
1.40	In statu quo. Drinks water greedily.
1.50	Continues depressed, but when irritated, movements are more lively.
2.	In statu quo.
2.10	Looks brighter. Head more continuously erect. Licks itself all over.
2.20	Nibbles oats occasionally.
2.30	Seems more itself again, though less bright than before injection.

Improvement continued more marked during succeeding half hour.

Exp. XVII.—Subcutaneous injection of ·9 cc. prepared extract into large, lively mouse.

10.55 a.m.	*Injection of ·9 cc.*
11.	In statu quo.
11.5	Has settled down in corner, with head depressed. Looks anxious. When irritated, moves about freely. Respiration appears quickened.

Exp. XVII.—*continued.*

11.15 a.m.	In statu quo. Respiration still quicker. Lies with head on table.
11.25	Occasionally fibrillary twitching over surface of body.
11.26	Slight general convulsive movement of trunk.
11.30	Very marked depression. Scarcely notices, when irritated.
11.35	Depression still deeper.
11.45	In statu quo.
12.	In statu quo. There has been no change.
12.10 p.m.	Depression appears less deep. Raises head occasionally.
12.15	Makes short movement forward, well co-ordinated. Speedily settles down to old condition.
12.20	Apparent improvement.
12.30	Accession of depression. Looks most anxious. During the last six minutes has had six general convulsive movements of abdomen.
12.45	In statu quo. No spasmodic movement.
1.	In statu quo.
1.10	Seems less depressed. Occasionally raises head.
1.20	Improvement continues.
1.30	Still further improvement. Occasionally makes voluntary movement forward.

During the next hour and a half the condition gradually improved. At 4 p.m., appeared more itself again.

Exp. XVIII.—Injection of 1·2 cc. prepared extract, subcutaneously, into medium-sized, lively mouse.

12.20 p.m.	*Injection of 1·2 cc.*
12.25	Looks frightened and anxious.
12.30	Much depressed. Lies in corner, with head resting on table. Makes little effort at voluntary movement. Respiration appears accelerated.

Exp. XVIII.—*continued.*

12.35 p.m.	Occasional convulsive movement of thorax and abdomen, like effort of vomiting. When irritated, makes slow, but co-ordinated movement forward. Speedily comes to rest again.
12.40	In statu quo. Respiration still quicker.
12.50	Depression grave, scarcely heeds irritation.
1.	In statu quo.
1.5	General convulsive movement of trunk every minute or two, which continue to come and go for eight to ten minutes.
1.15	Depression continues marked.
1.30	General quivering of surface of body from time to time.
1.45	In statu quo. Less of quivering.
2.	Appears rather less depressed. But pays no attention to oats placed beside it. Drinks water greedily.
2.15	In statu quo.
2.30	In statu quo.
2.45	Accession of depression, lies with chin on table, quite passive.
3.	In statu quo.
3.30	Slight signs of improvement. Raises head from time to time.
4.	Looks distinctly brighter. Makes occasionally voluntary movement forwards.

During the next hour or two, there was a slow but gradual improvement. No repetitions of convulsive movements. On the following morning it appeared as well as ever.

Exp. XIX.—Subcutaneous injection of 1·2 cc. prepared extract into large, lively mouse.

This experiment repeated, in almost every detail, that just recorded, and the record need not be given *in extenso*. The spasmodic movements of the trunk were most marked, about twenty minutes after injection.

Exp. XX.—Subcutaneous injection of 2 cc. prepared extract into medium-sized, lively mouse.

	Injection of 2 cc.
5.58 p.m.	
6.	In statu quo.
6.5	Looks anxious. Commencing signs of depression. Has settled to rest in corner.
6.10	Makes short forward movements, from time to time, as if in discomfort, looks frightened. Respiration hurried.
6.15	Depression deeper.
6.20	Scarcely heeds irritation. Spasmodic movements of trunk occur frequently, with occasional fibrillary twitching of surface of body.
6.25	In statu quo.
6.30	Depression intense. Lies absolutely passive, with head on ground.
6.45	In statu quo. There has been no change.
6.55	Occasionally makes restless movements forward for short distance, regular and co-ordinated. Speedily relapses into state of extreme depression.
7.	In statu quo. Spasmodic movements of trunk continue from time to time.
7.15	In statu quo.
7.30	In statu quo.
7.45	There has been no change.
8.	Depression even deeper.
8.15	Occasionally lifts head for second, but relapses again into old condition.
8.30	Rather less depressed.
8.45	Raises head and drinks water greedily. Oats not touched.
9.	No spasmodic movements for last half hour. Depression seems less deep.
9.10	Head more erect. Occasionally nibbles at oats.
9.20	Licks itself all over.
9.30	Continues to lick itself. Looks rather brighter.
9.45	In statu quo.
10.	Though brighter, still lies in corner, preferring to be left undisturbed.

Exp. XX.—*continued.*

On the following morning, looks brighter, but less lively than on previous day. During day, remains in same state eating little.

On third morning (9 a.m.), found dead.

P. M. EXAMINATION.—Rigor mortis not marked. Heart in diastole. Other organs seem normal. No sign of irritation at seat of injection.

Exp. XXI.—Subcutaneous injection of 2 cc. prepared extract into large, lively mouse, followed in two and three quarter hours by second injection of 1·6 cc. extract.

6 p.m.	*Injection of 2 cc.*
6.5	Commencing symptoms. During the first two and a half hours, the general course of the symptoms was very similar to that described under experiment XX. They need not therefore be detailed. The fibrillary and spasmodic movements were well marked. The depression was grave.
8.30	Head slightly raised. Appears rather brighter.
8.45	*Injection of 1·6 cc.*
8.50	Marked accession of symptoms. Spasmodic movements of abdomen very frequent.
8 55	Fibrillary twitching of surface of body constantly recurring. Lies flat on ground. Respiration very hurried.
9.	In statu quo.
9.5	Convulsive movements of head and upper extremities constantly repeated, occasionally of trunk generally. Lies as if dead. Respiration slower.
9.10	Convulsive movements continue.
9.15	In statu quo. Respiration slow and gulping.
9.20	Convulsive movements more general.

Exp. XXI.—*continued.*

9.30 p.m.	In statu quo.
9.40	Convulsive movements as before.
9.50	In statu quo.
10.	No improvement.
10.15	Lies extended and helpless, convulsive movements recurring every few seconds.
10.30	In statu quo.

On the following morning, convulsive movements absent and signs of slight general improvement. Still much depressed, and remains passive in corner, except when irritated. Looks most anxious. Declines food throughout day. No marked improvement observed, though convulsions remain absent. On third morning (10 a.m.), found dead.

P. M. EXAMINATION.—Rigor mortis not marked. Heart in diastole. Other organs seem normal. No irritation at seat of injection.

Exp. XXII.—Subcutaneous injection of 1·2 cc. prepared extract into lively *young* mouse (about half adult size).

10.52 a.m.	*Injection of 1·2 cc.*
	This was rapidly followed by similar symptoms to those detailed in expts. XX. and XXI., with a marked preponderance of the convulsive elements. Respiration was at first accelerated and then markedly slowed.
11.20	Lies helpless, as if dead. Frequently repeated spasmodic movements of trunk and upper extremity (fore limbs). Respiration gulping.
11.40	Respiration very slow. This state of extreme depression continued till
1. p.m.	Respiration and heart pulsation not to be made out.

P. M. EXAMINATION.—Heart in diastole, other organs seem normal. No irritation at seat of injection.

Other experiments were conducted on mice with exactly similar results.

Series 2.—Experiments to show the action of the extract on the heart, in so far as that is evidenced by alterations in the cardiac rate. (Frogs).

This series was undertaken with a view to determining the effects of the prepared extract on the heart, so far as that is evidenced by alterations in the cardiac rate. The experiments were conducted on frogs, with the heart exposed *in situ*. The frog was, in each instance, tied on its back in the usual way and a portion of the sternum carefully removed, so that the heart might be well seen, without a hernia of the abdomino-thoracic contents occurring. The injection needle was passed through the muscles of the thigh up into the posterior lymph sac, where the fluid was lodged as before. The temperature of the surrounding air was kept strictly fixed, while, with the exception of the "heart window," the frog was covered over with moist bibulous paper.

The six experiments (Exps. XXIII. to XXVIII.) are arranged in descending order, beginning with the largest dose (Exp. XXIII). As frequently happens, it will be noticed that the initial cardiac rate varied much. This must be borne in mind in judging of the effect. Of course, a fall of one or two pulsations per min., means more in a heart whose initial rate was 28 per min., than in one whose initial rate was 46 per minute. The several experiments may advantageously be compared with those of Series 1st, where corresponding quantities of the extract were used.

Exp. XXIII.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of medium-sized lively frog (R. temp.) Heart-rate 46 per min. Temperature of surrounding air = 15·5°C. (Cf. with this Exp. VI. Series 1.

Time.	Rate.	Remarks.
12.15 p.m.	46	+ ·9 cc.
12.23	49	Some struggling.
12.26	49	
12.30	48	
12.35	46	
12.40	44	
12.45	42	
12.55	42	
12.57 p.m.	36	Diastole lengthening, in proportion to systole.
1.	28	
1.3	24	
1.10	22	
1.15	21	
1.25	21	
1.30	21	
1.35	20	
1.40	19	Diastole even longer relatively.
1.50	20	
2.	20	
2.10	19	
2.20	20	
2.30	20	
2.35	19	
2.45	19	
3.	18	
4.	14	
5.30	14	In moribund state. Killed.

Exp. XXIV.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of large lively frog (R. temp.) Heart rate, 42 per min. Temp. of surrounding air, 13°C. (Cf. with this Exp. VIII., series 1).

Time.	Rate.	Remarks.
1.20 p.m.	42	+ ·9 cc.
1.27	42	
1.30	44	
1.40	44	
1.45	40	
1.50	34	
1.55	34	Diastole relatively lengthened
2.	32	
2.10	32	
2.20	31	
2.30	31	

(Experiment was here discontinued, with a view to observing certain contrast effects.)

Exp. XXV.—Injection of ·9 cc. prepared extract, undiluted, into posterior lymph sac of medium-sized lively frog (R. temp.) Heart rate, 33 per min. Temp. of surrounding air, 12·5°C. (Cf. with this Exp. VIII. 1).

Time.	Rate.	Remarks.
10.40 a.m.	33	+ ·9 cc.
10.50	34	
10.55	33	
11.	31	
11.5	31	
11.10	29	
11.15	28	
11.25	28	
11.35	27	Diastole lengthening in proportion to systole.
11.45	26	
11.55	25	
12.	25	

(Experiment was similarly discontinued at this point.)

Exp. XXVI.—Injection of ·7 cc. prepared extract, undiluted, into posterior lymph sac of medium-sized lively frog (R. temp.) Heart rate, 38 per min. Temp. of surrounding air 14° C.

Exp. XXVI.—*continued.*

Time.	Rate.	Remarks.
11.35 a.m.	38	+ ·7 cc.
11.45	42	Some struggling.
11.50	41	
11.55	38	
12.	37	
12.5 p.m.	31	Evidence of diastolic lengthening.
12.10	30	
12.15	28	
12.20	30	(Some struggling continued, rendered observation difficult, and experiment was therefore discontinued.)

Exp. XXVII.—Injection of ·6 cc. prepared extract, undiluted, into posterior lymph sac of medium-sized lively frog (R. temp.) Heart rate, 30 per min. Temp. of surrounding air 10·5°C. (Cf. with this Exp. V., series 1).

Time.	Rate.	Remarks.
11 a.m.	30	+ ·6 cc.
11.5	31	
11.7	31	
11.10	29	
11.15	28	
11.20	28	Some struggling interfered for minute or two with counting.
11.25	28	
11.30	26	
11.35	26	
11.40	25	
11.45	25	Diastolic lengthening.
11.50	24	
11.55	23	
12.	23	

Exp. XXVIII.—Injection of ·3 cc. prepared extract, undiluted, into posterior lymph sac of medium-sized lively frog (R. temp.) Heart rate, 28 per min. Temp. of surrounding air 10·5° C. (Cf. with this Exp. I., series 1).

Exp. XXVIII.—*continued.*

Time.	Rate.	Remarks.
11 a.m.	28	+ ·3 cc.
11.8	29	
11.10	30	
11.15	29	
11.20	28	
11.25	27	
11.30	26	
11.35	26	Relative lengthening of diastole.
11.40	25	
11.45	24	
11.50	25	
11.55	24	
12.	26	
12.10 p.m.	26	
12.15	26	
12.20	27	Continues restless, seems as if effect passing off.
12.30	27	
12.35	27	

Series 3.—Experiments to show how the general action of the extract is opposed by atropine.

A.—On Frogs (Exps. XXX.—XXXIV.).

In the first three experiments, the extract and the atropine were administered simultaneously, in different proportions. In Exp. XXXIII., the extract was exhibited first, and followed in half an hour by a suitable dose of atropine. In Exp. XXXIV., the exhibition of the extract was preceded half an hour by a dose of atropine.

Exp. XXX.—Injection of ·9 cc. prepared extract, + 1/200 milligramme Atrop. Sulphate into posterior lymph sac of large lively frog (R. temp.) Pupils medium.

1 p.m.	*Injection of ·9 cc. extr., + 1/200 mgm. Atr.*
1.5	Makes efforts at escape.
1.10	In statu quo.
1.15	Seems quieter rather.

Exp. XXX.—*continued.*

1.20 p.m.	In statu quo, head remains erect. Though quieter, re-acts readily, when irritated.
1.30	Still quieter. Jumps freely when irritated. If turned on back, rights itself.
1.40	In statu quo.
1.50	In statu quo.
2.	Slight depression appears passing off.

Exp. XXX.—*continued.*

2.15 p.m.	Condition still further improved.
2.30	More lively.
3.30	Appears as before injection.

N.B.—*Reflexes and pupils appear unaffected throughout.*

Here the symptoms produced by so large a dose as ·9 cc. of the extract (cf. Exps. VI., VII., and VIII.), are comparatively slight. The extract used, as in all the succeeding experiments, was one which, injected alone, gave most marked results. The great diminution in severity must therefore be referred to the Atropine, with which it was accompanied. It will be observed that the proportion of Atropine was not sufficient to completely neutralize the toxic principle in the extract.

Exp. XXXI.—Injection of ·7 cc. prepared extract, + 1/100, milligramme Atrop. Sulphat. into posterior lymph sac of medium-sized lively frog (R. temp.) Pupils medium.

9.35 a.m.	*Injection of ·7 cc. extr. + 1/100 mgm. Atrop.*
9.40	Jumps about freely.
9.45	Rather quieter. Less inclined to jump. But sits in ordinary position.
9.58	Continued disinclination for movement. Appears still quieter, but remains sitting with head erect. Pupils seem slightly increased in size.
10.	More lively.
10.15	Appears quite bright. Jumps about freely.
10.15	As before injection.

The symptoms were even slighter than in the last experiment. The increased proportion of Atropine appears to have almost completely neutralized the effects of the extract. In place of contraction of the pupil, there was observed (9.50 a.m.) slight dilatation.

Exp. XXXII.—Injection of ·6 cc. prepared extract + 1/66 milligramme Atrop. Sulphat. into posterior lymph sac of large lively frog (R. temp.) pupils medium.

9.25 a.m.	*Injection of ·6 cc. extr. + 1/66 mgm. Atrop.*
9.35	Remains lively.
9.45	In statu quo.
9.55	In statu quo.
10.15	As before. The frog was closely watched for two hours, with no discoverable alteration save slight dilatation of the pupil.

Here the symptoms produced by ·6 cc. (cf. Exp. V.) appeared neutralized by the 1/66 milligramme Atrop. Sulphat. injected simultaneously.

Exp. XXXIII.—Injection of ·9 cc. prepared extract, into posterior lymph sac of large lively frog (R. temp.) Pupils medium, followed in half an hour by injection of 1/66 milligramme Atrop. Sulphat.

10.30 a.m.	*Injection of ·9 cc. extract.*
10.35	No very marked change.
10.40	Distinctly quieter. Lies passive in corner, with head drooping.
10.45	In statu quo. When irritated, makes slow movement forwards, of crawling character.
10.50	Depression deeper; makes little resistance when turned on back. Lies indifferently on back or sides.
11.	In statu quo. Occasional spasmodic movement of limbs. Pupils seem less.
11.1	*Injection of 1·66 mgm. Atrop.*
11.5	In statu quo.
11.7	Head slightly raised. Makes short movement forwards.
11.10	For last two or three minutes has made repeated attempts at movement, still of crawling character. Head almost erect. Occasional return of spasmodic movements of limbs.

Exp. XXXIII.—*continued.*

11.15 a.m.	Movements are more frequent and more easily performed. Pupils slightly dilated.
11.25	Makes occasional effort at jump. If placed on back, will still remain in that position for some minutes.
11.35	Looks decidedly brighter. Improvement continues.
11.45	Jumps about. Offers resistance when attempt made to place on back. At once recovers itself.
11.50	Jumps about freely.
12.	Appears as before injection.

The results of this experiment were striking. During the first half hour, while the frog was under the influence of the extract, there was manifested an advancing depression, such as that seen in Exp. VII. Within ten minutes of the injection of the Atropine signs of improvement commenced, and these gradually developed till, within an hour of the later injection, the frog appeared itself again. The general course of the symptoms and the improvement, which occurred on the exhibition of Atropine, may advantageously be compared with those details in Exp. VII., where ·9 cc. prepared extract was made use of.

Exp. XXXIV.—Injection of ·9 cc. prepared extract into posterior lymph sac of large lively frog (R. temp.) Pupils medium, preceded, half an hour, by the injection of 1/100 milligramme Atrop. Sulphat.

10.25 a.m.	*Injection of 1/100 mgm. atrop. sulphat.*
10.30	Excited endeavours at escape.
10.35	Continued lively movements.
10.40	In statu quo. Throws itself against glass funnel in endeavour to escape.
10.45	Very lively. Pupils slightly dilated.
10.50	In statu quo.
10.55	*Injection of ·9 cc. prepared extract.*
11.	In statu quo.

Exp. XXXIV.—*continued.*

11.5 a.m.	Has become quieter. Jumps about less. Head erect.
11.10	Continues quieter. Sits at rest in corner, with head erect. When irritated, jumps about freely. Resists attempts to place it on back.
11.15	In statu quo.
11.20	Sits as before, but appears more depressed. Offers less resistance when it is turned on to back.
11.25	In statu quo. Will lie on back for moment or two if turned.
11.35	Appears rather less depressed. Will no longer lie on back.
11.45	Depression seems deeper. Lies on back for moment or two. When irritated, responds less. Pupils less.
12.	Looks brighter.
12.10 p.m.	Gradual improvement continues. Jumps about occasionally.
12.20	Pronounced improvement.
12.30	Jumps about freely.
11.45	Appears much itself again.

In this experiment, the endeavour was made, by a preliminary injection of atropine, to prevent the accession of the well-marked symptoms which had been found (Exps. VI., VII., and VIII.) to follow the exhibition of ·9 cc. extract. Having regard to the result of Exp. XXXII., a less amount of atropine was used than in that case. During the first half-hour succeeding the injection of the atropine, nothing was observed save slight general excitement, and a certain amount of pupillary dilatation. Within a quarter of an hour of the injection of the extract, those signs gave place to symptoms of slight depression. These latter continued more or less evident for an hour, but, as comparison with Exp. VII. will show, in addition to their later onset and much restricted duration, with much less intensity. The almost complete neutralization, as in Exp. XXXII., was obtained by the preliminary injection of a slightly larger amount of atropine (see Exps. in Series 4th.)

Series 3—*continued.*

B.—On Mice (Exps. XXXV., XXXVI.).

In both the following experiments, the extract and the atropine were given simultaneously. In each instance the extract was tested as to its physiological properties in other animals, apart from atropine.

The general results will be found to harmonize with those detailed in group *A*.

Exp. XXXV.—Injection of ·9 cc. prepared extract + 1/66 milligramme Atrop. Sulphat. subcutaneously into large lively mouse.

10.45 a.m.	*Injection of ·9 cc. extract + 1/66 mgm. atrop.*
10.50	In statu quo.
10.55	Continues lively. Runs about freely.
11.	Erect on hind legs, licking itself.
11.10	No signs of depression.
11.30	In statu quo.

Repeated observations were recorded throughout the day, but always negative, the mouse remaining undisturbed. On the following morning, it was found perfectly well. (Cf. with Exps. XVI. and XVII., Series 1st.)

On the forenoon of the second day the mouse received 1·8 cc. of the same extract, *without* atropine, when characteristic symptoms speedily supervened (cf. Exps. XVIII. and XIX., Series 1st), the mouse dying in twenty-four hours.

There can, therefore, be no doubt that the simultaneous injection of atropine sufficed to neutralize the toxic properties of the extract, which the second half of the experiment proves to have been well marked.

Exp. XXXVI.—Injection of 1·2 cc. prepared extract + 1/40 milligramme Atrop. Sulphat. subcutaneously into large lively mouse.

10.50 a.m.	*Injection of 1.2 cc. extr. + 1/40 mgm. atrop.*
10.55 a.m.	Runs about in lively fashion
11.	In statu quo. Licks itself all over. Nibbles oats freely.
11.10	In statu quo.
11.15	Seems quieter. Remains more constantly in one corner, unless irritated, when runs about as before. Head is erect.
11.20	Still quiet. Licks itself. Drinks water freely.
11.30	Depression appears to be passing off.
11.45	Appears as lively as before. Makes urgent endeavour to escape.

Throughout the afternoon, the mouse remained perfectly well. On the following day, the pupil was found to be much dilated, and the animal suffered from occasional spasmodic seizures. The symptoms were ascribed to an excessive action of the atropine.

A comparison of this experiment with experiments XVIII. and XIX., where the same quantity of the extract was injected, without the addition of atropine, is most instructive. In the latter, symptoms of grave depression were well marked for four or five hours, while in the experiment just described there was scarcely any deviation from the normal condition.

Series 4.—Experiments to show how the special action of the extract on the heart is opposed by atropine (frogs). (Exps. XXXVII.-XLIII.)

This last series was instituted, in view of the striking effects produced by the extract on the circulatory system, *i.e.*, on the cardiac rate in the frog as detailed in Exp. XXIII. to XXVIII. Some difficulty was experienced at first in approximately fixing the dose of atropine which would prove antagonistic to a measured quantity of the extract. The following are selected from a number of injections, the general results of which were similar. They may advantageously be compared with the experiments of Series 2nd, where the extract was administered alone.

As in Series 2nd the observations were made on frogs, with the heart exposed *in situ* (p. 23). The surrounding conditions were kept as constant as possible.

Exp. XXXVII.—Injection of ·7 cc. prepared extract, + 1/66 milligramme Atrop. Sulphat., into posterior lymph sac of large lively frog (R. temp.). Heart rate, 40 per min., temperature of surrounding air, 13·5°C. (Cf. with Exp. XXVI., series 2.) Pupil medium.

Time.	Rate.	Remarks.
11.40 a.m.	40	
11.43	40	+ ·7 *cc. extr.* + *1/66 mgm. Atrop.*
11.45	42	
11.47	43	
11.50	42	
11.55	40	
12.	40	
12.10	38	Pupils slightly dilated.
12.20	36	
12.30	36	
1.	36	
1.15	37	

Exp. XXXVII.—*continued.*

An interesting comparison may be made between this and Exp. XXVI., when a similar quantity of the extract was administered synchronously, under exactly similar conditions, but without Atropine. A still more striking contrast is obtained if reference be made to Exp. XXIII., where ·9 cc. extract was administered.

Exp. XXXVIII.—Injection of ·6 cc. prepared extract, + 1/66 milligramme Atrop. Sulphat., into posterior lymph sac of large lively frog (R. temp.). Heart rate, 44 per min., temp. of surrounding air, 15·5°C. Pupil small. (Cf. with Exp. XXVII., series 1.)

Time.	Rate.	Remarks.
12.35 p.m.	44	
12.40	44	

Exp. XXXVIII.—*continued.*

Time.	Rate.	Remarks.
12.40 p.m.		+ *6 cc. prepd. extr.* + *1/66 mgm. Atrop. Sulphat.*
12.45	44	
12.50	46	
12.53	48	
12.57	46	
1.	46	Pupil slightly dilated.
1.10	44	
1.20	43	
1.30	42	
1.40	42	
1.50	42	Frequently struggling.
2.	42	

So far as gauging results go, this experiment may be considered as concluded at 1.30 p.m., for in the succeeding half-hour no change is recorded. The total reduction in rate is, therefore, *two* beats per minute in a heart beating fairly rapidly. The result is in striking contrast with that obtained in Exp. XXVII., where, with the same dose of the extract, without atropine, there was produced a reduction of *six* beats per minute in a heart beating little more than half as rapidly.

Exp. XXXIX.—Injection of ·6 cc. prepared extract, + 1/66 milligramme Atrop. Sulphat. into posterior lymph sac of large lively frog (R. temp.). Heart rate, 38 per min., temp. of surrounding air, 12·5°C. Pupil medium. (Cf. with Exp. XXVII.)

Time.	Rate.	Remarks.
10.40 a.m.	38	
10.45	38	+ *·6 cc. prepd. ext.* + *1/66 mgm. Atr.*
10.50	40	
11.	40	

Exp. XXXIX.—*continued.*

Time.	Rate.	Remarks.
11.10 a.m.	40	Pupils slightly dilated.
11.20	40	
11.30	37	Frequent struggling made counting difficult.
11.40	40	
11.50	37	
12.	37	
12.10	36	
12.30	36	

The experiment was discontinued on account of prolonged struggling. The frog was removed after more than an hour and a half's treatment, living and active; while another frog, treated with the extract alone, for contrast, was evidently gravely depressed, when set free.

As in Exp. XXXVIII., the total decrease in cardiac rate amounts to two beats per minute, in a heart beating fairly fast. Cf. once more with Exp. XXVII.

Exp. XL.—Injection of ·6 cc. prepared extract, into posterior lymph sac of large lively frog (R. temp.), *preceded* (35 mins.) by injection of 1/66 mgm. Atrop. Sulphat. Heart rate, 29 per min., temp. of surrounding air, 13·5°C. Pupils medium.

Time.	Rate.	Remarks.
11 a.m.	29	Heart rate slow from beginning, probably owing to long continuance of severe weather, just prior to experiment.
11.7	29	+ *1/66 mgm. Atrop.*
11.15	29	
11.25	28	
11.35	28	
11.40	29	

Exp. XL.—*continued.*

Time.	Rate.	Remarks.
11.42 a.m.	29	Pupils somewhat dilated. + .6 cc. extr.
11.5	30	
11.55	30	
12.	30	
12.5 p.m.	29	
12.15	29	
12.25	29	Struggling from time to time.
12.30	30	
12.40	30	Pupil remains slightly dilated.
12.50	30	
1.	31	
1.10	32	Violent struggling and experiment discontinued.
1.30	32	Frog lively.

Here the heart rate, slow at first, was little affected during the first half-hour, when under the influence of atropine alone. During the hour succeeding the further injection of the extract, the rate, in place of diminishing, rather improved, so that the rate at 1.40 was three beats more than at 11.42. This is doubtless to be attributed to the atropine.

Exp. XLI.—Injection of ·6 cc. prepared extract, into posterior lymph sac of large lively frog (R. temp.), preceded (25 mins.) by injection of 1/66 milligramme Atrop. Sulphate. Heart rate, 34 per min. Temp. of surrounding air, 14°C. Pupils medium.

Time.	Rate.	Remarks.
12.40 p.m.	34	
12.45	34	+ 1/66 mgm. Atrop.
12.50	34	

Exp. XLI.—*continued.*

Time.	Rate.	Remarks.
1 p.m.	34.35	Frequent struggling. + ·6 cc. extr.
1.5	34	
1.15	34	
1.25	34	
1.40	34	
2.15	33	

Experiment was interrupted as the results were so constant. The frog was released lively, and killed. The results are very similar to those observed in the last experiment. There were no evident signs of depression throughout.

In the two concluding experiments, the administration of the extract was followed in half an hour (*circa*) by the exhibition of atropine. The doses of each were the same as in the two experiments which have just been under review.

Exp. XLII.—Injection of ·6 cc. prepared extract, into posterior lymph sac of large lively frog (R. temp.), followed in 35 mins. by injection of 1/66 milligramme Atrop. Sulphat. Heart rate, 34 per min. Temp. of surrounding air, 13·5°C. Pupils medium.

Time.	Rate.	Remarks.
11. a.m.	34	+ ·66 cc. extr.
11·10	35	
11.15	33	Frequent struggling.
11.20	32	
11.25	31	
11.30	29	Shows evident signs of depression. Lies more passive than usual. Diastole lengthening.
11.35	28	

Exp. XLII.—*continued.*

Time.	Rate.	Remarks.
11.40 a.m.	28.27	+ *1/66 mgm. Atr.*
11.45	30	
11.50	33	Struggling slightly.
11.55	32	
12.5 p.m.	32	
12.10	31	
12.20	31	
12.30	32	Improvement in character of systole.
12.50	32	
12.55	33	
1.5	33	
1.10	34	
1.20	34	Struggling.
1.30	34	Pupils dilated.

The experiment was now discontinued, and the frog released. When unbound, it jumped about in a lively fashion.

This experiment yielded most instructive results. The gradual decrease in heart rate is in keeping with Exps. XXIII. to XXVIII., Series 2nd. The condition of the frog up to 11.45 a.m. is similarly in harmony. The rapid improvement and uninterrupted return to the normal rate, after injection of atropine, is most striking.

Exp. XLIII.—Injection of ·6 cc. prepared extract into posterior lymph sac of large lively frog (R. temp.), followed in 35 mins. by injection of 1/66 milligramme Atrop. Sulphat. Heart rate, 33 per min. Temp. of surrounding air, 14°C. Pupils medium.

Exp. XLIII.—*continued.*

Time.	Rate.	Remarks.
12.30 p.m.	33	+ *·6 cc. extr.*
12.40	33	
12.45	34	
12.55	33	
1.	32	
1.5	31	Lengthening of diastole.
1.10	30	Frog lies more passive. + *1/66 mgm. atrop.*
1.15	30	
1.20	30	
1.25	32	
1.30	33	Improvement in character of systole.
1.35	33	
1.40	34	Frog appears brighter, struggles freely.
1.50	34	
2.	34	Pupils slightly dilated.
2.15	34	

The heart rate now continued constant and the experiment was discontinued. The frog was found lively when released.

The results of this experiment are almost identical with those of the last, and illustrate well the gradual decrease in heart rate during the unopposed action of the prepared extract (cf. Exps. XXIII. to XXVIII.), and the progressive increase which rapidly supervened after the exhibition of atropine.

The last two experiments were performed latest, and the comparatively perfect antagonism in respect of cardiac rate was obtained as a result of a large number of varying combinations. The general antagonism of the same doses is illustrated in another late experiment, recorded above, for convenience, as experiment XXXII.

(To aid comparison, it may be well to place side by side with one of this series the record of the experiment, already quoted as experiment XXIII., where ·9 cc. prepared extract, undiluted, was injected, under exactly similar conditions; the experiments being carried out side by side, and the same extract used, but in the latter *without the addition of atropine sulphate.*)

A. (**Exp. XXIII.**), *without atropine.*		*B.* (**Exp. XXXVIII.**), *extended record, with atropine.*	
Time.	Rate.	Time.	Rate.
12.15 p.m.	46 per min.	12.35 p.m.	44 per min.
		12.40	44
	+ ·9 cc. *prepd. ext.*		+ ·6 cc. *prepd. ext.* + 1/66 mgm. *atrop. sulph.*
12.23	49		
12.26	49	12.50	46
12.30	48	12.53	48
12.35	46	12.57	46
12.40	44	1.	46
12.45	42	1.8	44
12.55	42	1.12	44
12.57	36	1.15	42
1.	28	1.20	43
1.3	24	1.25	43
1.10	22	1·35	42
1.15	21	1.45	42
1.25	21	2.	42
1.30	21	2.5	40
1.35	20	2.10	39
1.40	19	2.20	39
1.50	20	2.30	39
2.	20	2.45	39
2.10	19	2.55	39
2.20	20	3.5	39
2.30	20	3.15	39
2.35	19	3.45	39
2.45	19	4.30	38
3.	18	5.	38
4.	14	5.30	38
5.30	14		

Here, the absence of any marked fall in the cardiac rate, when atropine sulphate was exhibited synchronously with the extract, is very striking, as contrasted with the serious fall recorded in the parallel column, where the extract was exhibited alone. These results seem to imply that the

depressant effect of the extract of which we have so conclusive proof in Exps. XXIII.—XXVIII., and which is thus neutralized by atropine, is produced through the medium of the cardio-inhibitory mechanism, and not through direct action on the cardiac ganglia or muscle. The value of this, from a practical point of view, will be discussed later.

II.—THERAPEUTIC.

With regard to the therapeutic aspect, I shall confine my remarks to the broad general lines, avoiding the discussion of details, which are easily appreciated when the guiding principles have been established. The outline may, it is hoped, have the double value of suggesting what must be the aim of our remedies at different stages of the disease, and what are the more hopeful avenues for laboratory and clinical investigation.

It must be premised, however, that what follows is in no way intended as subversive of tried, empirical remedies, but rather as a guide to the testing and sifting of these, and the assigning to each its proper place and value. As in the case of a skin affection, it is not likely that the physician will effect much if, in presence of a certain named disease, he prescribes merely what has come to be regarded as its specific remedy, apart from a consideration of the stage that has been reached and the special features which may be present, so, in each case of phthisis, failure is but courted, unless careful consideration be given to the stage of the disease and the factor or factors which, at the given stage, may more particularly be dealt with.

It is unnecessary, at this point, to recapitulate the views advanced as to the etiology of phthisis. For this reference may be made to the etiological division of the paper, and, in particular, to the general summary found at page 11. But it should be stated that the lines I am about to suggest have especial reference to what I believe to be by far the largest group of such cases—I mean those where phthisis seems to succeed a catarrhal condition of the respiratory passages.

Round this question most of the fighting has taken place, ever since the time when Laennec advanced the doctrine of the strictly tubercular basis of phthisis, while the opposition

school, led by Niemeyer, taught emphatically that "the consolidation and destruction of the lungs, which form the anatomical basis for consumption, are usually the products of inflammatory action, and the greater the quantity of cellular elements collected in the vesicles, and the longer the duration of the inflammation, so much the more readily will pneumonia lead to consumption."

It appears to me that, in the position which is adopted in the following pages, the meeting place of the two schools may be found, and their discrepancies harmonized.

Doubtless, in a large proportion of cases, there is, first of all, a general catarrhal condition of the respiratory passages, or, at least, a serious tendency to such catarrh. Certain individuals and families are peculiarly liable to this form of catarrh, as others, in turn, are more easily affected by certain other forms. This catarrhal condition may, or may not, be completely recovered from. So long as it involves the upper respiratory passages alone the condition is not immediately dangerous. The constant movement hinders the settlement, or at least prevents the development of the tubercle bacillus on the catarrhal secretion, while the probability of absorption is practically *nil*. But when once the ultimate vesicles are involved, more especially of those parts where there is comparative rest, the more or less stagnant muco-purulent secretion, which, there is reason to believe, is peculiarly well fitted as a cultivation medium, allows of the growth and development of the bacillus. This germinal energy on the part of the bacillus implies an elaboration of certain products. The arrangement of the secretion—now no longer purely catarrhal—in relation to the absorbent channels, permits the ready absorption of the elaborated products, which it has been the object of a large portion of this research to show to be poisonous in high degree. The grosser histological changes which ensue, such as the great increase in the fibrous elements and ulceration, are the result of the reaction of the tissues, and are to be regarded, I believe, as in part curative.

This seems to imply that a large proportion of the ordinarily

occurring cases of phthisis are at first simply catarrhal, and for such Niemeyer's doctrine of neglected catarrh holds good. On the other hand, it is extremely questionable whether a mere catarrh, even of the air vesicles, could lead *per se* to the graver conditions. But admit the presence of the bacillus, its falling on a suitable medium, and its subsequent development, with the elaboration of products, from cells already devitalised or in process of becoming devitalised, its penetration of the tissues, with the production of the peculiar histological accompaniments, and we have the foundation for the clinical features of a case of phthisis, as well as for those histological characteristics which Laennec and his school held to be the distinguishing element of the phthisical process.

The cases of general tuberculosis, where presumably there is a systemic infection through the blood, do not come under consideration at present.

From the therapeutic point of view, the facts before us suggest a general division of phthisis into three stages,—stages which, of course, are not always separable clinically, and all of which may be represented in different areas of the same chest. These stages are as follows :—

Stage I. *Catarrhal Stage proper*, previous to inoculation with the tubercle bacillus, *i.e.*, while the tubercle bacillus is threatening to invade (Pretubercular ?).

Stage II. *Stage of Invasion* by the tubercle bacillus, *i.e.*, when inoculation has been effected and the bacillus may be supposed to have begun developing in the catarrhal products, as in the cultivation medium outside the body. This stage naturally runs, without very evident limit, into the following.

Stage III. *Stage of Elaboration and Absorption, i.e.*, when the tubercle bacillus, in full growth, elaborates products which are slowly absorbed, and so general toxic effects are induced, with characteristic clinical features.[1]

What, then, are the therapeutic indications for each of these stages ?

[1] At the present stage of the discussion, I exclude advisedly all reference to the histological changes proceeding in the invaded lung.

I. Catarrhal Stage Proper.

Clinically, this is the stage of repeated "colds." The patient is very liable to such catarrhs, and does not throw them off so easily as the ordinary subject. Expectoration varies much in amount and character, according to the catarrh. Physical signs are not very characteristic, and may be entirely absent. The sputum ought not to contain the tubercle bacillus.

The therapeutic indications are essentially prophylactic. We have to recognise that the chief danger lies in the repetition and aggravation of these catarrhal attacks, and the tendency to localised stagnation of catarrhal products, with consequent liability to inoculation by the tubercle bacillus.

Thus, in addition to the institution of such tonic and general treatment as experience has proved most successful, we must adopt the utmost precautions against the occurrence of such catarrhs. All "colds," when frequently recurring, and of relatively long duration, must be viewed with suspicion and treated with attention and energy. Successful treatment implies the thoughtful study of the meteorological, climatic, and other conditions which are favourable to the production of catarrh, and the adoption of such, so far as may be, as are less favourable. Hence is seen the value of such work as that carried out by Bowditch, Buchanan, and others. Of extreme importance and suggestiveness is the line of work pursued by John Aitken, with reference to the number of dust particles in the atmosphere in different countries and cities, and at varying elevations. With the view of overcoming the tendency to stagnation, and the consequent danger of inoculation, well-regulated gymnastic and other exercise should be insisted on, and all means taken whereby the lazy part of the lung may be called into action, or congenital and other defects of chest architecture compensated. It is here that the compressed air bath has its appropriate sphere. Similarly, the question of relative degrees of atmospheric pressure will be of value in the selection of a climatic line of treatment. Lastly, the results of experi-

ments with ozone suggest that this gas possesses special activity in enabling the system to withstand the attacks of the bacillus.

It is further of interest, and not without value, to study the relation of tuberculosis pulmonum to other lung lesions. Thus, experience has shown that the supervention of tuberculosis on pulmonary emphysema is comparatively rare, and that the concurrence of tuberculosis and mitral disease is similarly uncommon; while, on the other hand, that tuberculosis frequently accompanies or follows conditions of lowered pressure in the pulmonary circulation. These facts seem to indicate a satisfactory *rationale* of the frequently observed coincidence of pulmonary phthisis and sedentary occupations. If, in addition to the sedentary life, with consequently diminished blood pressure, there is—as frequently co-exists—a more or less constant liability to the inhalation of impure air, containing dust and other noxious particles which are likely to induce or prolong catarrhal states of the air passages, all the necessary conditions are present for the successful lodgment and development of the tubercle bacillus.

Admitting that the gravest danger consists in the possible inoculation of the catarrhal products by the tubercle bacillus, we must direct attention to the natural history of that organism. Hence becomes apparent the *practical* value of all carefully collated facts with regard to the life history of micro-organisms. This is especially the province of bacteriology, and is sufficient justification of its recognition as a most important department of scientific inquiry. The more facts at our disposal with regard to a given pathogenic organism, the better directed our therapeutic endeavours will become. This implies such investigations as the consideration of the conditions of temperature, moisture, relative amounts of oxygen, ozone, &c., which are favourable or prejudicial to the development of the organism. The field may, in large degree, be overtaken by carefully instituted laboratory observations as to the varying conditions of growth, such, for example, as the fixing of ranges of temperature at which the organisms flourish best (Koch). The observation of the relative abundance of organisms in certain

districts and at certain elevations, initiated by Miquel, Pasteur, Koch, and others, is also suggestive of result, as is the further interesting inquiry into the greater or less frequency of disease in special districts, and under varying atmospheric and topographical conditions, with particular scrutiny of the relation between disease and milk and meat supplies. Lastly, the question of the chemical constitution of the micro-organisms may prove of value. Thus, comparatively recently, Hammerschlag has drawn attention to the striking difference between the chemical constitution of the tubercle bacillus and that of most other organisms, and staining methods appear to corroborate this observation.

It is impossible, meanwhile, to assign a definite value to each new fact in the life history of the tubercle bacillus. But, from the strictly therapeutic point of view, no such fact must be set aside as worthless.

II. Stage of Invasion.

Clinically, this is the stage when suspicion is often first aroused. The patient has, perhaps, been the subject of repeated catarrhs, and, probably, the "cold" is said to have taken hold of his chest. Stagnation of the catarrhal products has been more or less definitely established. It may not always be easy to detect well-marked physical signs, but frequently the breath sounds are distinctly altered, and, possibly, the percussion note. There may or may not be audible abnormal auscultatory accompaniments. The temperature is variable. The general condition of the patient varies much with the degree of advancement of this stage, which merges insensibly into the succeeding one. The sputum contains the tubercle bacillus in greater or less abundance.

The therapeutic indications lie in the direction of exhibiting tonic and other general remedies for the relief of the catarrh on the lines already indicated, but more especially in the adoption of such measures as are likely to render the inspissated secretion and altered tissues less suitable media for the growth of the tubercle bacillus.

The problem comes to be—Granting that the more or less stagnant catarrhal product has allowed of the deposit and growth of the organism, can we, by any means, so modify the constitution of the nutrient media as to make them unfit for, or noxious to, the further development of the bacillus?

This may theoretically be done in one of two ways. On the one hand, we may hope to modify their constitution in some general way, as was referred to in speaking of other cultivation media (p. 3). For example, the dropping of the nitrogenous elements in a given medium may be expected to prevent the growth in it of such organisms as demand for their development the presence of nitrogen. Such a method, as applied to the subject before us, is surrounded with difficulties, and an approach to their proper solution can only be obtained by a careful comparison of the different media on which the tubercle bacillus may be artificially cultivated, and the consideration of how far we may be able to influence the character of the catarrhal secretion and the infested tissues through the system. In this connection the interesting researches of Nocard and Roux must be recalled. These observers have shown that the addition of 6-8 per cent. glycerine to the culture medium favours the growth of the tubercle bacillus remarkably. Growth is much more rapid and abundant, and the form of the individual organism undergoes a change. It has also been maintained that organisms thus reared are less virulent and also less hardy. Other observers have shown that the addition of sugar acts similarly.

Closely akin to this line of thought is the question, whether by rendering the catarrhal secretion more liquid, in addition to aiding free expectoration, we might not possibly prevent the full development of the organism. From this point of view the question of the relation existing between pulmonary tuberculosis and conditions of lowered or of increased blood-pressure assumes considerable significance.

On the other hand, we might hope to introduce some foreign element, which might be so noxious to the tubercle bacillus, or so disturbing to the necessary conditions of its develop-

ment, as, more or less completely, to hinder the possibility of its growth. This, I take it, is the basis of the so-called "antiseptic" line in the treatment of phthisis. (The introduction of the idea of "septicity" is misleading.) Whether the agent adopted be used by inhalation or, what *a priori* appears more evidently efficacious, by diffusion through the pulmonary circulation and subsequent exhalation, the result aimed at should be the same—*not so much the actual death of the bacilli present* (and this is an error into which not a few have fallen in speaking of such a line of treatment), *but rather the saturation of the medium with such agents as may be supposed or may be proved experimentally to be prejudicial to the vital activity of the organisms*,—for, as has been said, "the life of the bacillus is difficult, easily discouraged by unfavourable circumstances, like an aphis by an easterly wind."

Here, again, we must fall back on experiment, a most captivating line of experiment, pregnant with results. By means of variation in the cultivation medium, but, more especially, by the addition in certain proportions to the media of various substances, it may be shown that the growth of organisms can be materially hindered, if not entirely prevented. Already there is a large body of recorded experimental work on this point, conducted by Koch and many other observers, and more especially as regards the tubercle bacillus, by Sormani and Brugnatelli, Yersin, and others. Thus Sormani and Brugnatelli have shown that lactic acid, camphor, bromide of ethyl, naphthol (α and β), turpentine, creasote, chloride of palladium, and bichloride of ethyl, when mixed in varying proportions with phthisical sputum, neutralize its virulence, as tested by subcutaneous injection, while a number of other bodies have a distinct power of attenuation. Similarly Yersin has subjected tubercular matter to the influence of certain disinfecting agents, and after such treatment has attempted to obtain cultivations from the same. His results showed that carbolic acid, absolute alcohol, ethereal solution of iodoform, æther, corrosive sublimate, thymol, and salicylic acid availed, in varying strengths, to destroy all the

tubercular organisms. But in every instance, the necessary concentration was so great, or the length of exposure was so prolonged, as to afford little hope for the *direct* practical application of any of the agents he used. The more exact experimental method, however, from the present point of view, is the attempt to so *modify* a recognised cultivation medium, *e.g.*, inspissated blood serum, by the addition of bodies similar to those cited, as to destroy its powers of sustaining the life of the bacillus. For logically it is *unnecessary to seek for a body which would actually kill the parasite present.* Such a body might be expected to have equally, if not more, deleterious influence on the cell elements of the host. We have rather to *seek a means of so altering the medium as to disturb the necessary condition of growth.*

This appears to me the rationale of the undoubted benefit to be derived at this stage from the group of bodies represented by eucalyptol, turpentine, terebene, copaiba, (and, perhaps, creasote), whose volatile principles are, in part, excreted through the respiratory surfaces. From some of these bodies we doubtless have the double advantage of a benignant action on the mucous surfaces, and of impregnation of the catarrhal secretion with products which prove antagonistic to the development of the tubercle bacillus. From this point of view, I have for the last two or three years used the pure oil of the eucalyptus globulus with much advantage in all stages of phthisis. The oil—and its purity must be guaranteed—is given internally in doses of 10-30 minims three or four times daily, or it may be given—and I often combine both methods —by intra-tracheal injection in the proportion of 20-30 per cent. eucalyptus oil in olive oil or other bland menstruum. By the prolonged use of the combined method I have been able to record cases of cure, while, in almost every instance, a striking amelioration of symptoms has resulted.

For the past three years I have made trial of a great variety of remedies of this class by the intra-tracheal method. As the result of an experience covering many thousand injections, I am of opinion that this method of introducing drugs is easy, safe, and efficacious in a large proportion of cases.

The passage of the curved syringe involves little more difficulty than the introduction of the laryngeal mirror. The injection causes, in scarcely a single instance, subjective sensations of an unpleasant nature. Laryngeal spasm is extremely rare, and even cough occurs seldom. On the other hand, the patients very speedily report a feeling of relief, accompanied by agreeable sensations. If in cases complicated by exacerbations of dyspnœa, *e.g.*, by night, the injections are made at bedtime, the patient quickly falls into an easy and undisturbed sleep. So simple, indeed, is the method, that I have been led to make use of the respiratory mucous membrane for the absorption of a variety of remedies, when the gastro-intestinal tract was not available.

As a routine injection fluid, in phthisis, I have been latterly using the following:—

℞.

Chloroform, *pur.*, ...	♏. xxx.
Balsam Peru, ...	ʒij.
Olei Eucalypti, ...	ʒij.
Olei Ricini ad., ...	ʒj.

Sig.

30 minims for a dose once or twice daily.

With few exceptions, our patients have made excellent progress in the manner I have just indicated. Very soon after the treatment is commenced the patient's cough is relieved, the expectoration is diminished, the bacilli frequently become fewer, and gradually the patient's skin assumes a more healthy look, regaining its natural soft and moist character, night sweats cease, and there is a marked gain in weight. Thus, I have had to record the gain of 18 lbs. in nine weeks.

In a similar category, come such substances as iodine (so much extolled by Germain Sée), and the allied iodoform and group of iodides, much of whose virtue doubtless depends on the free iodine exhaled by the respiratory mucus surfaces. We may also suppose that salicylic acid and the salicylates, which are of undoubted benefit in certain stages of phthisis, act

in a similar way. For salicylates have been separated from the sputum; while it has been proved experimentally that salicylic acid added in definite strength to a fluid containing bacteria, prevents their development, and added in greater concentration, destroys such organisms when abundantly present. It is unnecessary here to give an extended list of such substances. Those cited will serve as illustrations of the point under consideration.

A passing reference may, perhaps, be made to the suggestion for the treatment of phthisis, by the exhibition of sulphuretted hydrogen *per rectum*. The system which was introduced by Bergeon of Lyons, and was more or less successfully practised in many hospitals, proceeds on the principle we have been discussing. The sulphuretted hydrogen absorbed by the intestinal mucous surfaces, is carried to the lungs and there exhaled. In process of exhalation, it doubtless modifies the excreting surface and the catarrhal products and tuberculous tissue on which the bacilli are developing. The method, therefore, occupies the same platform as others already referred to, while its success has not been such as to justify more special encomium. It is interesting to note, in view of the present argument, that Bergeon and other observers have recorded that successful results were chiefly obtained in cases of early phthisis, *i.e.*, presumably cases in the second stage according to our present classification.

Before passing to the consideration of the third stage, a word or two of criticism must be offered regarding the so-called method of Bacterio-therapy suggested by Cantani. It consists in the systematic inhalation of a spray produced by a fluid containing bacteria in suspension. It is based on the principle of the survival of the fittest, and it has been affirmed, that under such treatment the bacillus tuberculosis falls before the bacterium termo. But the recorded results are doubtful, and certain facts may be cited in direct opposition. Thus, Koch inoculated numbers of highly tubercular guinea pigs with the bacillus anthracis, and at the *post mortem* examination, some time later, discovered both forms of bacillus in abundance. He

has also described a case where, in a highly tubercular subject, with abundant evidence of bacilli, a large number of the vessels were found plugged with emboli formed by micrococci. Further, Kirstein has detailed a case of tuberculosis of the urinary organs, where both the tubercular bacillus and more ordinary forms of bacteria were present in numbers, side by side. Babes too has demonstrated the frequent occurrence of the tubercle bacillus along with the streptococcus pyogenes and the diplococcus pneumoniæ (Fränkel). Then, experimentally, it can be shown that the vital action of the tubercle bacillus continues undisturbed though kept for a considerable time in putrefying sputum, in presence of various forms of bacteria and micrococci, as is proved both by inoculation and by the reaction to staining fluids. On the clinical side, Sormani and others have closely followed Cantani's method, without obtaining satisfactory results.

In passing to the consideration of Stage III., it must be premised that clinically we must not expect to be able to make a sharp line of demarcation where Stage II. closes and Stage III. begins. To a considerable extent they merge into one another, and, during a long period of the clinical history, they doubtless run side by side in different portions of the affected lungs. Hence we must expect to have to make use of two lines of the therapeutic treatment simultaneously. This is made intelligible, if it be clearly understood *why* each line is adopted.

III. Stage of Absorption.

Clinically, this is the stage of *phthisis* proper. There is progressive emaciation, and more or less anorexia. Night sweating is profuse. The temperature is irregular, and the general condition of the patient most unsatisfactory. The physical signs are, in general, well marked. The sputum contains the tubercle bacillus, and, on *post-mortem* examination, the bacillus is found infiltrating the tissues.

It is to this stage especially that much of the experimental side of the foregoing paper has reference.

The therapeutic indications cover, in part, those lines which

have been suggested for Stage II. This is the stage, further, where such questions as the advisability of cutting down on the diseased portions of lung, and the instituting of free drainage present themselves. The establishment of the factory where the elaboration of the toxic products is carried on having taken place, we may, by this means, endeavour to cleanse these foci, and thus, in part, prevent further mischief. But this is not always practicable, and the method, though thoroughly sound in principle, is, meanwhile, in its infancy and we cannot hope immediately for its general adoption.

But if we accept the deductions drawn from the experimental part of this research, it becomes our duty to seek to discover and make use of such remedies as may be proved antagonistic to the evolved products which are in course of absorption. The experimental results of Series 3 and 4 point clearly to Atropine as one of these. For the estimation of the scope of this antagonism, reference must be made to the detailed results (*supra*).

Since the date of the earlier experiments I have adopted clinically for this stage the exhibition of large doses of belladonna. Hitherto the use of belladonna has been limited to its occasional exhibition as an anti-hydrotic, and this largely on empiric grounds. But I believe its action to be much more general. I have, therefore, exhibited it at frequent intervals by day, and it is kept up until physiological action is evidently induced. I generally prescribe it in tincture or extract form, sometimes as the sulphate of atropine; but the form is, doubtless, of little importance.

The effects in a number of instances have been most striking. The patients have expressed themselves as feeling much better. The appetite has improved, the weight has increased, there has been less sweating, and the temperature has fallen. Unfortunately in dispensary practice successful results are, in part, neutralized by the impossibility of obtaining the necessary co-operation of some of the lines of treatment for Stages I. and II.

My experience has been corroborated by the results obtained by a number of physicians who have, at my suggestion, made trial of the remedy, and kindly reported to me.

It is interesting, historically, to know that Trousseau had, long ago, expressed the belief that atropine exerted a specific action on the lung tissue in phthisis. More recently Bartholow records 'that he has observed cases of phthisis which appear to him to have been remarkably improved by the continued use of this remedy.' It may be that the beneficial effects which these distinguished clinicians detected were really due to such an antagonism as it has been the object of the latter part of my experiments to establish.

We may safely express the belief that other drugs will be found effective in the same lines, so that an important line is opened up for experimental and clinical research.

In bringing this sketch to a conclusion, I think it well to add that I do not regard the etiological deductions from the experimental part, or the therapeutic principles, in any sense complete or fixed. But they appear to me justified by the facts; and they afford, at least, a rational basis for a good working hypothesis. They are submitted in the hope that they may be the means of giving clearer definition to our conception of phthisis, and greater precision to our methods of treatment.

Appendix.

(*A.*) *The Nature of the Toxic Product.*

It must be admitted that, in the extract used for injection, we have probably to deal with more than one product. This view is confirmed by the result of the further attempts at separation which I have instituted.

The concentrated extract, as obtained by the method already described (p. 6), is a slightly turbid liquid of yellowish-grey colour, which, on standing for twenty-four to thirty-six hours, separates into a clearer supernatant layer of lighter colour, and a somewhat granular deposit of varying shades of white or yellow. The extract has a characteristically mawkish odour; its taste is sour. With litmus paper it gives the acid reaction.

On adding a few drops of Fehling's solution to 2 c.c of the extract a faint violet tint is obtained, such as is got in the ordinary test for peptones; with tannic acid the diluted extract gives an abundant, flaky white precipitate; with neutral solution of platinic chloride, a faint yellowish precipitate, not distinctly crystalline; with phosphomolybdic acid, an amorphous yellow precipitate, turning later to greenish-blue; with iodine test solution (iodine dissolved in solution of potassic iodide), a flaky brownish precipitate; and with Meyer's mercuric iodide solution, a slight amorphous white precipitate. The amorphous precipitate yielded by the last test, assumes, after standing for some time, a faintly crystalline form.

The colour reaction on the addition of Fehling's solution may be taken as indicating the probable presence of a peptone. This is not contra-indicated by the reactions obtained with tannic acid and with Meyer's solution (while not necessarily corroborated thereby). Further, it is most probable, from the origin of the extract and the methods employed for its preparation, that a peptone should be formed. The sputum is a body rich in albuminous compounds, and the process to which it is subjected in the preparation is, in many points, analogous to

that of slow digestion at blood heat. Moreover, it is recognised that peptones may be possessed of toxic properties.

On the other hand, it is evident that all the reactions cannot be ascribed to the presence of such peptone. More especially, the precipitate obtained with platinic chloride indicates the presence of another body, possibly alkaloidal. And with this view some of the other tests are in consonance.

With the view of further clearing up this point, the attempt was made to separate the peptone presumably present from the other supposititious principle. Having regard to the comparative non-solubility of peptones in absolute alcohol and their perfect non-solubility in ether, a modification of the Stas-Otto system of separation was adopted. The sputum, carefully collected and measured, as for the other method (see p. 6), is slowly treated with three volumes of rectified spirit (the addition of the spirit being made approximately *guttatim*, with a view to the fullest separation of the elements of the sputum). Tartaric acid is added until the reaction of the whole is distinctly acid. It is then digested for twenty-four hours, at a gentle heat, in a Florence flask, with a long tube carried from the neck to admit of condensation of the alcoholic vapour. At the end of this period, the whole is carefully filtered more than once, and the filtrate evaporated slowly, at a gentle heat, over a water bath. When reduced to the consistency of a fairly concentrated extract it is again filtered. The clear fluid passes through, leaving a considerable deposit, which is washed with rectified spirit, the washings being in turn filtered and added to the first filtrate. The united filtrate, reduced still further by slow evaporation, is then treated with absolute alcohol, which, added slowly, produces a fresh copious precipitation. The clear alcoholic solution having been carefully decanted and filtered, is once more evaporated. What remains after evaporation, having been ascertained to be acid, is then dissolved in a small quantity of distilled water and shaken up with ether. The ether is finally removed and allowed to evaporate.

On evaporation of the ether, a relatively large amount of a yellowish amorphous body is left in the capsule. After the

watery solution has been thus treated with ether two or three times (each succeeding ethereal evaporation affording less of the product), it is rendered distinctly alkaline by the addition of ammonia, and treated with ether as before. On evaporation, the ether leaves traces of an amorphous body of rather paler yellow colour. Subsequent extraction, by means of chloroform and benzole, gives only negative results.

The body separated from the acid solution is of yellow colour and amorphous. On standing in the cold, however, for some days, there appear within it traces of imperfect crystallisation. It is possessed of a peculiarly penetrating odour. The body separated from the alkaline solution is much less in amount, of a paler yellow colour, and amorphous. Its smell is similar. The amorphous masses, carefully treated with weak hydrochloric acid, afford, after slow evaporation in the cold, further manifestation of crystallisation, though hardly sufficiently defined to allow of accurate description.

The amorphous body has been submitted to several tests. It gives a yellow precipitate of crystalline character with platinic chloride, a flaky brown with iodine solution, an abundant, flaky precipitate of yellowish colour, turning to greenish with phosphomolybdic acid, a light whitish precipitate with Meyer's solution, and an abundant flaky precipitate with tannic acid. These reactions are most evidently obtained from the product of the acid ethereal extract, but are also obtainable with that from the alkaline. It is thus possible that the two bodies are identical, a certain proportion passing off from the alkaline solution, because unremoved in the acid ethereal extract (cf. the possibility of removing atropine and veratrine in part in ethereal solution from an acid fluid, though the major part is removed only when the fluid is made alkaline). The possibility that here also we have to deal with two distinct bodies must not be lost sight of.

With the body obtained thus comparatively pure, both in its amorphous form and when combined with acid, as a hydrochlorate, various experiments were made on frogs. These, so far, have been restricted in number, owing to the great expendi-

ture of time and material involved in the preparation of a sufficiently large quantity. It may be stated, however, that the results obtained were distinct, and in close correspondence with those detailed as having been produced by the more readily accessible extract. Their striking identity points to the conclusion that the physiological effects are *especially* dependent on the body, whose characters have just been discussed, and not on the peptone, whose presence has been indicated. So much may be granted without assuming too much or unduly excluding the efficient action of the peptone.

The body whose separation has thus been attempted, and whose presumptive effects have been detailed, would thus seem to bear closest resemblance to the group of ptomaines.

(*B.*) *An Improved Method for the Demonstration of Bacilli in Tubercular Sputum, and for the obtaining of Pure Cultivations from such Sputum.*

Communicated to the Medico-Chirurgical Society of Edinburgh, July 1886.

Considerable difficulty frequently attends the methods generally used for the demonstration of the bacillus tuberculosis in phthisical sputum. This depends more particularly on (*a*) the comparative paucity of the micro-organisms in relation to the cell elements in certain cases; and (*b*) the extremely tenacious character of the mucus, which renders it no easy matter to obtain a suitably thin and equal layer on the cover glass. Similar difficulty (and in some cases greater) attends the attempt to utilise the sputum for the purpose of obtaining "pure cultivations." Even where the micro-organismal elements are present in large number, rendering the sputum a peculiarly favourable source, the extreme cohesiveness of the secretion offers no small hindrance to successful inoculation.

Both these difficulties are obviated by the adoption of the following method, while, in doubtful cases, the demonstration of the presence of the bacillus is rendered more certain.

The principle is that of separation and concentration. These are obtained by a double process of incubation and slow precipitation or deposition. In so far, it is thus practically the same principle as that followed in the examination of the urine for tube casts and other solid elements, the peculiar character of the secretion, in the case of the sputum, making the carrying out of the principle more complicated. The process of incubation, further, probably introduces an additional factor of value, to which reference will be made later.

Method.—The sputum of from 12 to 24 hours is carefully collected and kept as free from the access of foreign particles as may be. It is then exposed in a beaker or flask to the influence of a gentle moist heat for 24 hours, or longer, if necessary. The mouth of the vessel should be protected with a thin piece of gauze, which permits the free entrance of air and moisture, while excluding the grosser impurities. The continuous exposure described may be most conveniently carried out in a Koch's steam steriliser, as used for bacteriological work, but this is not essential. The temperature best suited for the process ranges from 36°-39° C. (cf. Koch's optimum range for the growth of a pure cultivation.)

At the close of 24 hours the appearance of the sputum has altered considerably. The heavier cell elements have separated more or less fully, and become deposited at the bottom of the vessel, while above this is a uniform layer of comparatively clear fluid. The clear supernatant fluid may be decanted, leaving the thicker deposit. This latter is no longer cohesive, but may be spread with ease and exactness as a uniform layer on the cover glass. After drying and staining in the usual way it is mounted, and microscopic examination reveals the advantage of the procedure. The heavier cell elements in their descent have carried down the micro-organismal elements, so that the drop contains these in relatively increased numbers. I have found in some instances that the consequent multiplication has been five times or more. Further, the uniform distribution of the elements enables us more easily to gauge the relative abundance of the bacilli. The absence of the

tenacious quality admits, too, of greater certainty in the carrying out of the manipulative method necessary for the obtaining of a cultivation.

While the apparent increase in the number of bacilli is, in large part, to be attributed to the precipitation and resulting concentration, I am inclined to think that there is also an absolute increase, which is to be regarded as due to the process of incubation to which the sputum was subjected. For, in addition to the evident multiplication, I have been able to trace what appear to be signs of more active proliferation. This may appear opposed to the observation that the growth and development of the tubercle bacillus in artificial cultivation, *e.g.*, on inspissated blood serum, is slow. On the other hand, it appears to lend support to the view that has been suggested in the foregoing paper, that possibly the sputum is a medium peculiarly well fitted for its development. The grounds for this suggestion have been sufficiently discussed in the text. It may fairly be added, however, that the extreme abundance of the organisms in the sputum in certain cases would seem to support the idea. In addition to the signs of an increased number, the grouping and variety in size and form are striking.

(*C.*) *Clinical Method for Staining the Tubercle Bacillus.*

All my earlier work was carried out by the use of the Ehrlich Weigert method, which will always hold its own for beauty of demonstration. But for clinical purposes, where rapidity is a matter of considerable importance, it has its disadvantages. For the last year or two, therefore, I have adopted what is generally known as the Ziehl Neelsen method, which is rapid and sure. The whole process may be gone through, and a judgment passed, where a positive result is obtainable, in five minutes. When the first observations are negative, the process is, of course, longer, and the adoption of the plan described in Appendix (*A*) may prove necessary.

It may be convenient, if I add a note of the procedure by the Ziehl Neelsen method.

Two solutions are used :—

I.	Fuchsin, ...	1 part.
	5 °/₀ solution of carbolic acid in distilled water, .	100 parts.
	Absolute alcohol, ...	10 parts.
II.	Methyl blue, ...	1 part.
	25 °/₀ solution of sulphuric acid in distilled water,	100 parts.

Prepare and gently dry a cover glass preparation of the sputum. Float downwards for *two* minutes in solution I., which should be warmed till steam rises. Rinse in clean water, and place in solution II. for *one* minute. Rinse again in cold water. Dry upper surface, and mount on slide in glycerine or Farrant's medium.

For ordinary clinical purposes, this is sufficient. If a more perfect demonstration be desired, this can be obtained, at the expense of rapidity, by drying, clearing up with oil of cloves, or cedar oil, and alcohol, and mounting in balsam or similar medium.

WELLCOME LIBRARY INSTITUTE